# ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF DEXIBUPROFEN IN BULK AND TABLET DOSAGE FORM BY RP-HPLC AND HPTLC METHOD

***Dissertation work submitted to***
***The Tamil Nadu Dr. M.G.R. Medical University, Chennai***
***in partial fulfillment for the award of degree of***

**MASTER OF PHARMACY**

**(PHARMACEUTICAL ANALYSIS)**

Submitted by

**R.RAJESWARI**

***Reg No. 26106427***

Under the Guidance of

**Institutional Guide**

**Dr. W.D. Sam Solomon M.Pharm., Ph.D**

**Professor & Head**

**Department of Pharmaceutical Analysis**

**RVS College of Pharmaceutical Sciences**

**Sulur, Coimbatore.**

**Industrial Guide**

**Mr. Shaik Mohamed Noor**

**Senior Manager**

**Shasun Pharmaceuticals Ltd**

**Puducherry.**

**May 2012**

**DEPARTMENT OF PHARMACEUTICAL ANALYSIS**

**R.V.S. COLLEGE OF PHARMACEUTICAL SCIENCES**

**Sulur, Coimbatore – 641402.**

# CERTIFICATE

This is to certify that the research project work entitled "**Analytical Method development and Validation for Estimation of Dexibuprofen in bulk and tablet dosage form by RP-HPLC and HPTLC method**" is a bonafide work of **Ms.RAJESWARI.R (Reg.No.26106427)** carried out in **Shasun Pharmaceuticals Limited, Puducherry** under my guidance and has completed to my fullest satisfaction for partial fulfillment of the award of degree of **Master of Pharmacy in Pharmaceutical Analysis**, **RVS College of Pharmaceutical Sciences, Sulur, Coimbatore,** which is affiliated to **The Tamilnadu Dr.M.G.R.Medical University, Chennai**. This work is original and contributory.

Place : Coimbatore

Date :

**Dr. W.D. SAM SOLOMON** M.Pharm.,Ph.D
Professor & Head
Department of Pharmaceutical Analysis
R.V.S. College of Pharmaceutical Sciences
Sulur, Coimbatore.

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Place : Coimbatore

Date :

**Dr. R. VENKATANARAYANAN** M.Pharm.,Ph.D

Principal

R.V.S. College of Pharmaceutical Sciences

Sulur, Coimbatore.

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**INTERNAL EXAMINER**

**Place:**

**Date:**

**EXTERNAL EXAMINER**

**Place:**

**Date:**

## *ACKNOWLEDGEMENT*

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***Date:*** **R.RAJESWARI**

***Place: Coimbatore***

# 1. INTRODUCTION

A drug may be defined as a substance meant for diagnosis, cure, prevention, or treatment of diseases in human beings or animals or for alternating any structure or function of the body of human being or animals.

Analytical method development and validation play important roles in the drug discovery, development, and manufacture of pharmaceuticals.

This presentation will discuss the development and validation of analytical method (High performance liquid chromatography (HPLC), High performance thin layer chromatography (HPTLC)) for drug products.

The number of drugs introduced into market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one. Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities (resulting in their withdrawal from the market), and development of patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures of these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs.

**Basic Criteria for New Method Development of Drug Analysis[1]:**

- The drug may not be official in any pharmacopoeias.
- A proper analytical procedure for the drug may not be available in the literature due to patent regulations.
- Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients.
- Analytical methods for the quantitation of the drug in biological fluids may not be available.

**Introduction to HPLC method of analysis for drug:**

Most of the drugs dosage forms can be analysed by HPLC method because of the several advantages like rapidity, specificity, accuracy, precision and ease of automation in this method.

Some of the advantages are:

- Speed (analysis can be accomplished in 20 minutes or less),
- Greater sensitivity (various detectors can be employed),
- Improved resolution (wide variety of stationary phases),
- Reusable columns (expensive columns but can be used for many analysis),
- Ideal for the substances of low volatility,
- Easy sample recovery, handling and maintenance,
- Instrumentation tends itself to automation and quantitation (less time and less labour),
- Precise and reproducible,
- Calculation are done by integrator itself,
- Suitable for preparative liquid chromatography on a much larger scale.

Chromatographic methods are commonly used for the quantitative and qualitative analysis of raw materials, drug substances, drug products and compounds in biological fluids[2].

**TYPES OF CHROMATOGRAPHY[3-6]:**

Chromatography is a technique by which the components in a sample, carried by the liquid or gaseous phase, are resolved by sorption- desorption steps on the stationary phase.

**A. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC):**

High performance liquid chromatography separation based on interaction and differential partition of the sample between the mobile liquid phase and the stationary phase. The commonly used chromatographic methods are divided into the following groups,

1. Normal phase
2. Reverse phase
3. Chiral
4. Ion exchange
5. Ion pair
6. Affinity
7. Size exclusion

1. **NORMAL PHASE CHROMATOGRAPHY:**

In the normal phase mode, the stationary phase is polar and the mobile phase is nonpolar in nature. In this technique, nonpolar compounds travel faster and are eluted first. This is because of the lower affinity between the nonpolar compounds and the stationary phase. Polar compounds are retained for longer times because of their higher affinity with the stationary phase. These compounds, therefore take more times to elute. Normal phase mode of separation is therefore, not generally used for pharmaceutical application because most of the drug molecules are polar in nature and hence take longer time to elute.

2**. REVERSE PHASE CHROMATOGRAPHY**:

Reversed phase mode is the most popular mode for analytical and preparative separations of compound of interest in chemical, biological, pharmaceutical, food and biomedical sciences. In this mode, the stationary phase is nonpolar hydrophobic packing with octyl or octa decyl functional group bonded to silica gel and the mobile phase is polar solvent. An aqueous mobile phase allows the use of secondary solute chemical equilibrium (such as ionization control, ion suppression, ion pairing and complexation) to control retention and selectivity. The polar compound gets eluted first in this mode and nonpolar compounds are retained for longer time. As most of the drugs and pharmaceuticals are polar in nature, they are not retained for longer times and hence elute faster. The different columns used are octa decyl silane (ODS) or $C_{18}$, $C_8$, $C_4$, etc; (in the order of increasing polarity of the stationary phase).

**3. CHIRAL PHASE CHROMATOGRAPHY:**

Separation of the enantiomers can be achieved by on chiral stationary phases by formation of diastereomers via derivating agents or mobile phase additives on achiral stationary phases. When used as an impurity test method, the sensitivity is enhanced if the enantiomeric impurity elutes before the enantiomeric drug.

**4. ION EXCHANGE CHROMATOGRAPHY:**

In ion exchange chromatography, the stationary phase contains ionic groups like $NR_3^+$or $SO_3^-$ which interact with the ionic groups of the sample molecules. This is suitable for the separation of charged molecules only. Changing the pH and salt concentration can modulate the retention.

**5. ION PAIR CHROMATOGRAPHY:**

Ion pair chromatography may be used for the separation of ionic compounds and this method can also substitute for ion exchange chromatography. Strong acidic and basic compounds may be separated by reversed phase mode by forming ion pairs with suitable counter ions. This technique is referred to as reversed phase ion pair chromatography.

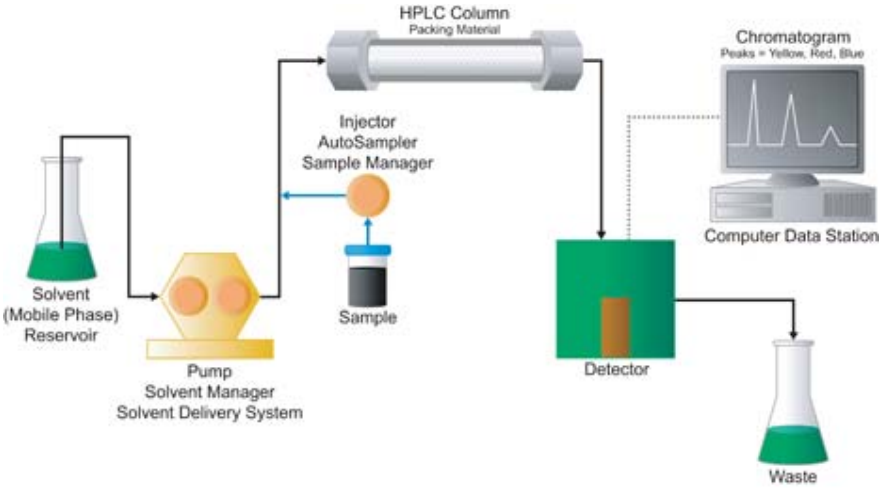
**6. AFFINITY CHROMATOGRAPHY:**

Affinity chromatography uses highly specific biochemical interactions for separation. The stationary phase contains specific groups of molecules which can absorb the sample if certain steric and charge related conditions are satisfied. This technique can be used to isolate proteins, enzymes as well as antibodies from complex mixtures.

**7. SIZE EXCLUSION CHROMATOGRAPHY:**

Size exclusion chromatography separates molecules according to their molecular mass. Largest molecules are eluted first and the smallest molecules last. This method is generally used when a mixture contains compounds with a molecular mass difference of at least 10%. This mode can be further subdivided into gel permeation chromatography (with organic solvents) and gel filtration chromatography (with aqueous solvents).

**SCHEMATIC DIAGRAM OF HPLC EQUIPMENT[7]**




In HPLC the mobile phase acts as a carrier for the sample solution. A sample solution is injected into the mobile phase through the injector port. As the sample solution flows through

a column with the mobile phase, the components of that solution migrate according to the non-covalent interaction of the compound with the column; determine the degree of migration and separation of components contained in the sample. The mobile phase can be altered in order to manipulate the interactions of the sample and the stationary phase. There are several types of elution namely: isocratic, gradient, and polytypic.

**IN ISOCRATIC ELUTION:**

Isocratic elution compounds are eluted using constant mobile phase composition all compounds begin migration through the column at onset. However each migrates at a different rate, resulting in faster or slower elution rate. This type of elution is both simple and inexpensive, but resolution of some compounds is questionable and elution may not be obtained in a reasonable amount of time.

**IN GRADIENT ELUTION:**

Gradient elution different compounds are eluted by increasing the strength of the organic solvent. The sample is injected while a weaker mobile phase is being applied to the system. The strength of the mobile phase is later increased in increments by raising the organic solvent fraction, which subsequently results in elution of retained components. This is usually done in a stepwise or linear fashion.

**COMPONENTS OF HPLC[8-11]:**

- Solvent delivery system
- Pump
- Sample injection system
- Column
- Detector
- Recorder

**A. SOLVENT DELIVERY SYSTEM:**

A mobile phase is pumped pressure from one or several reservoir and flows through the column at a constant rate. For normal phase separation eluting power increases with increasing polarity of the solvent but for reversed phase separation, eluting power decreases with increasing polarity.

A degasser is needed to remove dissolved air and other gases from the solvent. Special grades of solvents are available for HPLC and these have been purified carefully in order to remove absorbing impurities and particulate matter to prevent these particles from damaging the pumping or injection system or clogging the column.

**PUMPS:**

The pump is one of the most important component of HPLC, since its performance directly affects retention time, reproducibility and detector sensitivity.

Three main types of pumps are used in HPLC to propel the liquid mobile phase through the system.

**1. DISPLACEMENT PUMP**: It produces a flow that tends to independent of viscosity and back pressure and also output is pulse free. But is possesses limited capacity (250 ml).

**2. RECIPROCATING PUMP:** It has small internal volume (35 to 400µl), their high output pressure (up to 10,000 psi) and their constant flow rates. But it produces a pulsed flow.

**3. PNEUMATIC OR CONSTANT PRESSURE PUMP:** They are pulse free, suffer from limited capacity as well as a dependence of flow rate on solvent viscosity and column back pressure. They are limited to pressure less than 2000 psi.

**B. SAMPLE INJECTION SYSTEM:**

Insertion of the sample onto the pressurized column must be as a narrow plug so that the peak broadening attributable to this step is negligible. The injection system itself should have no dead (void) volume.

There are three important ways of introducing the sample into injection port.

**1. LOOP INJECTION:**

In which, a fixed amount of volume is introduced by making use of fixed volume loop injector.

**2. VALVE INJECTION:**

In which, a variable is introduced by making use of an injection valve.

**3. COLUMN INJECTION:**

In which, a variable volume is introduced by means of a syringe through a septum.

**C. CHROMATOGRAPHIC COLUMN:**

The column is usually made up of heavy glass or stainless steel tubing to withstand high pressure. The columns are usually 10-30 cm long and 4-10 mm inside diameter containing stationary phase at particle diameter of 25 µm or less. Columns with an internal diameter of 5mm give good results because of compromise between efficiency, sample capacity, and the amount of packing and solvent required.

**COLUMN PACKING:**

The packing used in modern HPLC consist of small, rigid particles having an arrow particle size distribution. There are three main types of column packing in HPLC.

1. **POROUS, POLYMERIC BEDS:**

Porous, polymeric beds based on styrene divinely benzene co-polymers used. For analytical purpose these have now been replaced by silica based, packing which are more efficient and more stable.

**2. POROUS LAYER BEDS:**

Consisting of a thin shell (1-3µm) of silica or modified silica on an spherical inert core (e.g. glass). After the development of totally porous micro particulate packing, these have not been used in HPLC.

**3. TOTALLY POROUS SILICA PARTICLES (dia. 10 µm):**

These packing have widely been used for analytical HPLC in recent years. Particles of diameter 20µm are usually dry packed. While particles of diameter 20µm are slurry packed in which particles are suspended on a suitable solvent and the slurry so obtained is driven into the column under pressure.

**D. DETECTORS:**

The function of the detector in HPLC is to monitor the mobile phase as it merges from the column. Detectors are usually of two types.

**1. BULK PROPERTY DETECTORS:**

It compares overall changes in a physical property of the mobile phase with and without an eluting solute. E.g. Refractive index, Dielectric constant or Density.

2. **SOLUTE PROPERTY DETECTORS:**

It responds to a physical property of the solute which is not exhibited by the pure mobile phases. E.g. UV absorbance, Fluorescence or diffusion current. Such detectors are about 1000 times more sensitive giving a detectable signal for a few nano grams of sample.

**METHOD DEVELOPMENT BY HPLC[12-14]:**

Method for analysing drug dosage form can be developed, depend upon the

- Nature of the sample
- Molecular weight
- Polarity
- Ionic character
- Solubility

In general one begins with reversed phase chromatography, when the compounds are hydrophilic in nature with many polar groups and are water soluble. The organic phase concentration required for the mobile phase can be estimated by gradient elution method. For aqueous sample mixtures, the best way to start is with gradient reverse phase chromatography. Gradient can be started with 5-10 % organic phase in the mobile phase and the organic phase concentration (methanol or acetonitrile) can be increased up to 100% within 30-45 min.

The various parameters for method development.

1. Mode of separation
2. Selection of stationary phase
3. Selection of mobile phase
4. Selection of detector

**1. SELECTION OF MODE OF SEPARATION:**

In reverse phase mode, the mobile phase is comparatively more polar than the stationary phase. For the separation of polar or moderately polar compounds, the most preferred mode is reverse phase. The nature of the analyte is the primary factor in the selection of the mode of separation. A second factor is the nature of the matrix.

**2. SELECTION OF STATIONARY PHASE / COLUMN:**

Selection of the column is the first and the most important step in method development. The appropriate choice of separation column includes three different approaches

1. Selection of separation system
2. The particle size and the nature of the column packing.
3. The physical parameters of the column i.e. the length and the diameter.

Some of the important parameters considered while selecting chromatographic columns are

- Length and diameter of the column.
- Packing material
- Shape of the particles
- Size of the particles
- Percentage of carbon loading
- Pore volume
- Surface area
- End capping

The column is selected depending on the nature of the solute and the information about the analyte. Reversed phase mode of chromatography facilitates a wide range of columns like dimethyl silane ($C_2$), butyl silane ($C_4$), octyl silane ($C_8$), octadecyl silane ($C_{18}$), base deactivated silane ($C_{18}$) BDS phenyl, cyanopropyl (CN), nitro, amino etc. $C_{18}$ was chosen for

this study. Generally longer columns provide better separation due to higher theoretical plate numbers. As the particle size decreases the surface area available for coating increases. Column with 5µm particle size give the best compromise of efficiency, reproducibility and reliability. In this case the column selected had a particle size of 5 µm and a internal diameter of 4.6mm

Peak shape is equally important in method development. Columns that provide symmetrical peaks are always preferred while peaks with poor asymmetry can result in

- In accurate plate number and resolution measurement
- Imprecise quantitation
- Degraded and undetected minor bands in the peak tail
- Poor retention reproducibility

A useful and practical measurement of peak shape is peak asymmetry factor and peak tailing factor. Peak asymmetry is measured at 10% of full peak height and peak tailing factor at 5%. Reproducibility of retention time and capacity factor is important for developing a rugged and repeatable method. A column which gives separation of all the impurities and degrades from each other and from analyte peak and which is rugged for variation in mobile phase shall be selected.

**3. SELECTION OF MOBILE PHASE:**

The primary objective in selection and optimization of mobile phase is to achieve optimum separation of all the individual impurities and degrades from each other and from analyte peak.

In liquid chromatography, the solute retention is governed by the solute distribution factor, which reflects the different interactions of the solute-stationary phase, solute-mobile phase and the mobile phase-stationary phase. For a given stationary phase, the retention of the given solute depends directly upon the mobile phase, the nature and the composition of which has to be judiciously selected in order to get appropriate and required solute retention. The mobile phase has to be adopted in terms of elution strength (solute retention) and solvent selectivity (solute separation).

Solvent polarity is the key word in chromatographic separations. Since a polar mobile phase will give rise to low solute retention in normal phase and high solute retention in reverse

phase LC. The selectivity will be particularly altered if the buffer pH is close to the pKa of the analytes. The solvent strength is a measure of its ability to pull analyte from the column. It is generally controlled by the concentration of the solvent with the highest strength .The following parameters, which shall be taken into consideration while selecting and optimizing the mobile phase.

- Buffer
- pH of the buffer
- Mobile phase composition

**3.1. Buffer:**

Buffer and its strength play an important role in deciding the peak symmetries and separations. Some of the most common buffers are

- Phosphate buffers prepared using salts like $K_2HPO_4$, $K_2HPO_4$, $NaH_2PO_4$, $Na_2HPO_4$, etc.
- Phosphoric acid buffer using $H_3PO_4$
- Acetate buffers – Ammonium Acetate, Sodium Acetate, etc.
- Acetic acid buffers using $CH_3COOH$.

The retention time also depend on the molar strength of the buffer. Molar strength is increasingly proportional to retention time.

**3.2. pH of the buffer:**

pH plays an important role in achieving the chromatographic separations as it controls the elution properties by controlling the ionization characteristics. To maintain the pH of the mobile phase in the range of 2.0 to 8.0 as most columns does not withstand to the pH which are outside this range. This is due to the fact that the siloxane linkage are cleaved below pH 2.0, while the pH value above 8.0 silica may dissolve.

**3.3. MOBILE PHASE COMPOSITION:**

Most chromatographic separations can be achieved by choosing the optimum mobile phase composition. This is due to the fact that fairly large amount of selectivity can be achieved by choosing the qualitative and quantitative composition of aqueous and organic portions. Most widely used solvent in reverse phase chromatography are methanol and acetonitrile.

Experiments were conducted with mobile phases having buffers with different pH and different organic phases to check for the best separations between the impurities.

A mobile phase which gives separation of all the impurities and degrades from the each other and from analyte peak and which is rugged for variation of both aqueous and organic phase by at least ± 0.2% of the selected mobile phase composition.

**SYSTEM SUITABILITY TESTS FOR CHROMATOGRAPHIC METHODS[15-17]:**

System suitability is the checking of a system to ensure system performance before or during analysis of unknowns.

System suitability parameters and Recommendations:

| SL.NO | PARAMETERS | RECOMMENDATIONS |
|---|---|---|
| 1. | Capacity factor (k´) | The peak should be well resolved from other peaks and the void volume, generally k´ 2.0. |
| 2. | Repeatability | RSD / = 1% for N / = 5 is desirable. |
| 3. | Relative retention | Not essential as long as the resolution is stated. |
| 4. | Resolution ($R_s$) | $R_S$ of 2 between the peak of interest and the closest eluting potential interfere (impurity, excipient, degradation product, internal standard, etc. |
| 5. | Tailing factor (T) | T of / = 2 |
| 6. | Theoretical plates (N) | In general should be 2000 |

The parameters that are affected by the changes in chromatographic conditions are:

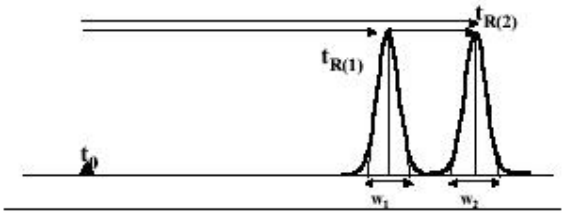
- Resolution ($R_S$),
- Capacity factor (k´),
- Selectivity (a),
- Column efficiency (N),
- Peak asymmetry factor ($A_S$).

**1. RESOLUTION ($R_S$):**

Resolution is the parameter describing the separation power of the complete chromatographic system relative to the particular components of the mixture. The resolution of two neighbouring peak is defined as the ratio of the distance between two peak maxima. It is the difference between the retention times of two solutes divided by their average peak width. For baseline separation, the ideal value of $R_S$ is 1.5. It is calculated by using the formula,

**EXPERIMENTAL RESOLUTION**

$$Rs = \frac{t_{R(2)} - t_{R(1)}}{1/2\ (w_1 + w_2)}$$




Where $Rt_1$ and $Rt_2$ are the retention times of components 1 and 2

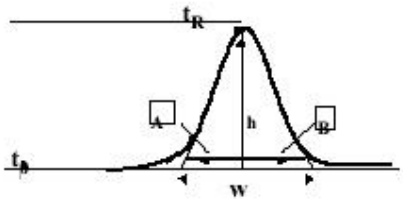
$W_1$ and $W_2$ are peak width of components 1 and 2.

**2. CAPACITY FACTOR (K´):**

Capacity factor is the ratio of the reduced retention volume to the dead volume. Capacity factor, k´, is defined as the ratio of the number of molecules of solute in the stationary phase to the number of molecules of the same in the mobile phase. Capacity factor is a measure of how well the sample molecule is retained by a column during an isocratic separation. The ideal value of k´ ranges from 2-10. Capacity factor can be determined by using the formula.

**RETENTION FACTOR or CAPACITY RATIO**

$$k' = \frac{t_R - t_0}{t_0} \qquad k' = \phi \frac{C_s}{C_m}$$



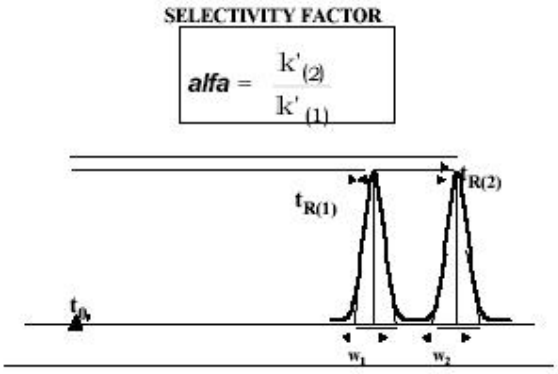
Where, $t_R$ = Retention volume at the apex of the peak (solute ) and

$t_o$ = void volume of the system.

**3. SELECTIVITY (A) :**

The selectivity (or separation factor) is a measure of relative retention of two components in a mixture. Selectivity is the ratio of the capacity factors of both peaks, and the ratio of the capacity factors of both peaks, and the ratio of its adjusted retention times. Selectivity represents the separation power of particular adsorbent to the mixture of these particular components.

This parameter is independent of the column efficiency, it only depends on the nature of the components, elute type, and eluent composition and adsorbent surface chemistry. In general, if the selectivity of two components is equal to 1, then there is no way to separate them by improving the column efficiency. The ideal value of a is 2.




It can be calculated by using formula,

$a = V_2 – V_1 / V_1 – V_0 = K_1´ / K_2´$

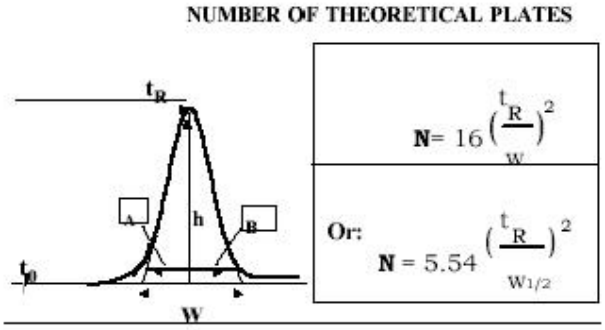
Where, $V_0$ = The void volume of the column,

$V_1$ and $V_2$ = The retention volumes of the second and the first peak respectively.

**4. COLUMN EFFICIENCY / BAND BROADENING (N) :**

Efficiency of a column is measured by the number of theoretical plates per meter. It is a measure of band spreading of a peak. Similar the band spread, higher is the number of theoretical plates, indicating good column and system performance. Columns with N ranging

from 5000 to 100,000 plates/ meter are ideal for a good system. Efficiency is calculated by using the formula,

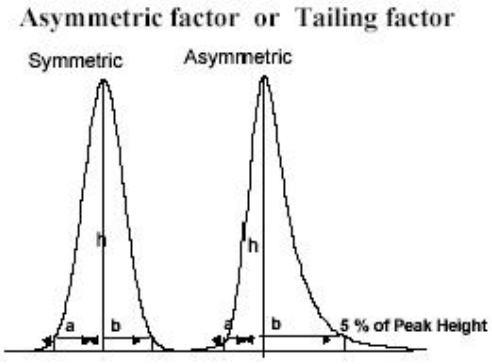



Where, $t_R$ is the retention time and

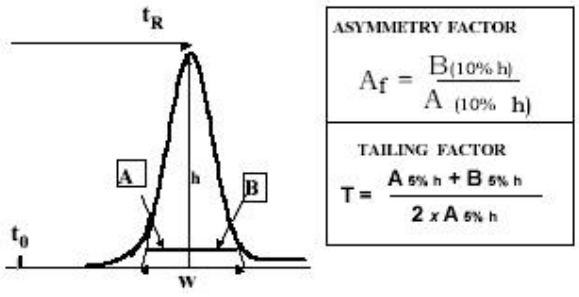
W is the peak width.

**5. PEAK ASYMMETRY FACTOR ($T_F$):**

Peak asymmetry factor can be used as a column performance. The peak half width 'b' of a peak at 10% of the peak height, divided by the corresponding front half width 'a' gives the asymmetry factor. For a well packed column, an asymmetry factor of 0.9 to 1.1 should be achievable.




Asymmetric factor or Tailing factor



## INTRODUCTION TO HPTLC METHODS OF ANALYSIS FOR DRUGS[18-20]

HPTLC (High Performance Thin Layer Chromatography) is a well known and versatile separation method which shows a lot of advantages in comparison to other separation techniques.

| | |
|---|---|
| Layer of Sorbent | 100µm |
| Efficiency | High due to smaller particle size generated |
| Separations | 3-5 cm |
| Analysis time | Shorter migration distance and the analysis time is greatly reduced |
| Solid support | Wide choice of stationary phases like silica gel for normal phase for reversed phase modes |
| Development chamber | New type that require less amount of mobile phase |
| Sample spotting | Auto sampler |
| Scanning | Use of UV/Visible/Fluorescence scanner scans the entire chromatogram qualitatively and quantitatively and the scanner is an advanced type of densitometer. |

## FEATURES OF HPTLC:

1. Simultaneous processing of sample and standard-better analytical precision and accuracy less need for internal standard.
2. Several analysts work simultaneously
3. Lower analysis time and less cost per analysis.
4. Low maintenance cost.
5. Simple sample preparation-handle samples of divergent nature.
6. No prior treatment for solvents like filtration and degassing.
7. Low mobile phase consumption per sample.
8. No interference from previous analysis-fresh stationary and mobile phases for each analysis-no contamination.

9. Visual detection possible-open system.
10. Non UV absorbing compounds detected by post-chromatographic derivatization.

**STEPS INVOLVED IN HPTLC:**

1. Selection of chromatographic layer
2. Sample and standard preparation
3. Layer pre-washing
4. Layer pre-conditioning
5. Application of sample and standard
6. Chromatographic development
7. Detection of spots
8. Scanning
9. Documentation of chromatic plate

**SELECTION OF CHROMATOGRAPHIC LAYER:**

- Precoated plated-different support materials- different sorbents available.
- 80% of analysis – silica gel GF. Basic substances, alkaloids and steroids aluminium oxide. Amino acids, dipeptides, sugars and alkaloids- cellulose.
- Non-polar substances, fatty acids, carotenoids, cholesterol – RP2, RP8 and RP 18.
- Preservatives, barbiturates, analgesic and phenothiazine - Hybrid plates - RPWF254s.

**SAMPLE AND STANDARD PREPARATION:**

- To avoid interference from impurities and water vapours.
- Low signal to noise ration- Straight base line- Improvement of LOD.
- Solvents used are Methanol, Chloroform: Methanol (1:1), Ethyl acetate: Methanol (1:1), Chloroform: Methanol: Ammonia (90:9:1), Methylene chloride: Methanol (1:1), 1% Ammonia or 1% Acetic acid.
- Dry the plates and store in dust free atmosphere.

**ACTIVATION OF PRE-COATED PLATES:**

- Freshly open box of plates do not require activation.
- Plates exposed to high humidity or kept on hand for long time to be activated.
- By placing in an oven at 110-120°C for 30’ prior to spotting.

- Aluminium sheets should be kept in between two glass plated and placing in oven at 110-120°C for 15 minutes.

**APPLICATION OF SAMPLE AND STANDARD:**

- Usual concentration range is 0.1-1µg/µl.
- Above this causes poor separation.
- Linomat IV (automatic applicator) - nitrogen gas sprays sample and standard from syringe on TLC plates as bands.
- Band wise application – better separation – high response to densitometer.

**SELECTION OF MOBILE PHASE:**

- Trial and error
- One's own experience and literature

**NORMAL PHASE**

- Stationary phase is polar
- Mobile phase is non polar
- Non polar compounds eluted first because of higher affinity with the stationary phase

**REVERSE PHASE**

- Stationary phase is non polar
- Mobile phase is polar
- Polar compounds eluted first because of lower affinity with stationary phase
- Non–polar compounds retained because of higher affinity with the stationary phase.
- 3-4 component mobile phase should be avoided.
- Multi component mobile phase once used not recommended for further use and solvent composition is expressed by volumes (v/v) and sum of volumes is usually 100.
- Twin trough chambers are used only 10 – 15 ml of mobile phase is required
- Components of mobile phase should be mixed introduced into the twin – trough chamber.

**PRE- CONDITIONING (CHAMBER SATURATION):**

- Un saturation chamber causes high Rf values
- Saturated chamber by lining with filter paper for 30 minutes prior to development.
- Uniform distribution of solvent vapours.
- Less solvent for the sample to travel.
- Lower Rf values.

**CHROMATOGRAPHIC DEVELOPMENT AND DRYING:**

- After development, remove the plate and mobile phase is removed from the plate.
- To avoid contamination of lab atmosphere.
- Dry in vacuum desiccator.
- Avoid hair drier.
- Essential oil components may evapourate.

**DETECTION AND VISUALIZATION:**

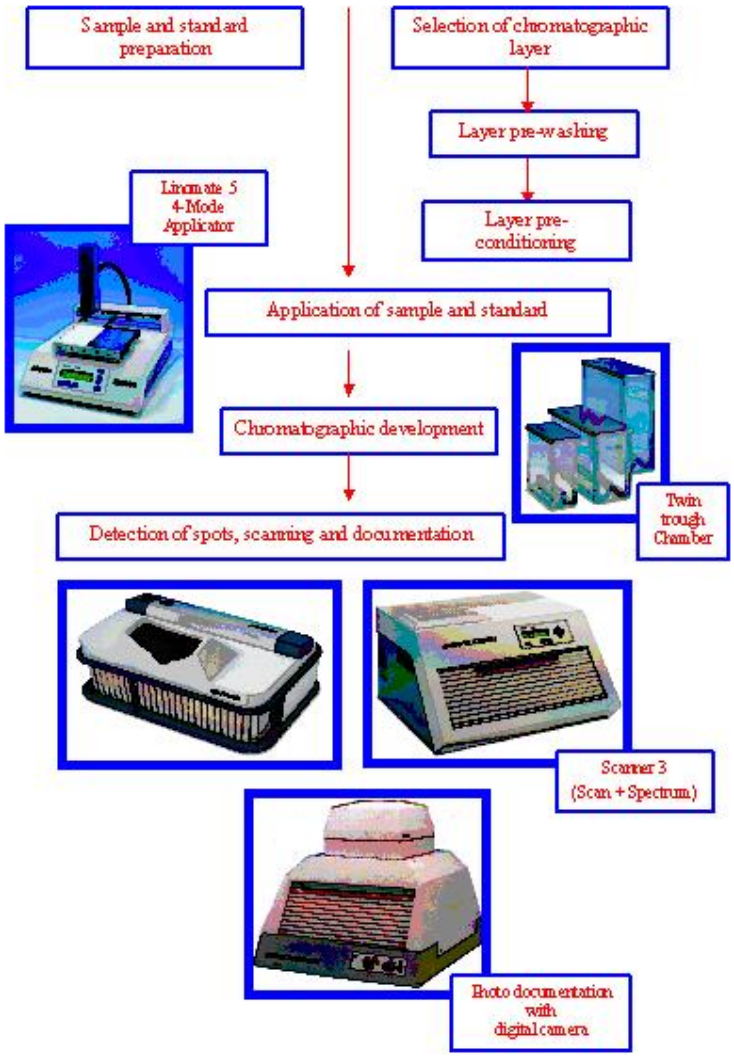
- Detection under UV light is first choice.
- Non-destructive.
- Spots of fluorescent compounds can been.
- Fluorescent stationary phase is used.
- Silica gel GF.
- Non UV absorbing compounds like ethambutol, dicyclomine etc.
- Dipping the plates in 0.1 % iodine solution.
- When individual component does not respond to UV.
- Derivatisation required for detection.

**QUANTIFICATION:**

- Sample and standard should be chromatographed on same plate.
- After development chromatogram is scanned.
- Camag TLC scanners III scan the chromatogram in reflectance or in transmittance mode by absorbance or by fluorescent mode.
- Scanning speed is selectable up to 100 mm/s.
- A spectrum recording is fast.
- 36 tracks with up to 100 peak windows can be evaluated.

- Calibration of single and multiple levels with linear or non- linear regressions are possible when target values are to be verified such as stability testing and dissolution profile single level calibration is suitable
- Statistics such as RSD or CI report automatically.
- Concentration of analyte in the sample is calculated by considering the sample initially taken and dilution factors.

**Fig.No: 2 SCHEMATIC PROCEDURE FOR HPTLC**



**HPTLC Method design and development**

Set the analytical objective first that may be quantification or qualitative identification or optimization of analysis time before starting HPTLC. Method for analyzing drugs dosage forms by HPTLC demands primary knowledge about the

- Nature of the sample,
- Namely, structure,
- Polarity,
- Volatility,
- Stability,
- Solubility.

An exact recipe for HPTLC, however, also same like HPLC cannot be provided because method development involves considerable trial and error procedures. The most difficult problem usually is where to start, with what kind of mobile phase.

Selection of stationary phase is quite easy that is to start with silica gel which is reasonable and nearly suits all kinds of drugs. Mobile phase optimization is carried out by using three level techniques. First level involves use of neat solvents and then by finding some solvents which can have average separation power for the desired drugs. Second level involves decreasing or increasing solvent strength using hexane or water for respective purposes. Third level involves trying of mixtures instead of neat solvents from the selected solvents of first and second level which can further be optimized by the use of modifier like acids or bases.

Analytes are detected using fluorescence mode or absorbance mode. But if the analytes are not detected perfectly than it need change of stationary phase or mobile phase or need the help of pre or post chromatographic derivatization. Optimization can be started only after a reasonable chromatogram which can be done by slight change in mobile phase composition. This leads to a reasonable chromatogram which has all the desired peaks in symmetry and well separated.

Parameters that are affected by the changes in chromatographic conditions are:

- Retention factor ($R_f$),
- Peak purity,

**1. RETENTION FACTOR ($R_F$):**

Retention factor is defined as the amount of separation due to the solvent migration through the sorbent layer as shown in the formula. It depends upon the time of development and velocity coefficient or solvent front velocity.

$$R_f = \frac{\text{Migration distance of substance}}{\text{Migration distance of solvent front from origin}}$$

**2. PEAK PURITY:**

The null hypothesis "these spectra are identical" can in this case (purity) with two sided significance. During the purity test the spectrum taken at the first peak slope is correlated with the spectrum of peak maximum [r(s, m)] and the correlation of the spectra taken at peak maximum with the one from the down slope or peak end [r(m, e)] which is used as a reference spectra for statistical calculation. An error probability of 1% only is rejected if the test value is greater than or equal to 2.576.

**VALIDATION OF ANALYTICAL METHOD[21-23]:**

Validation is an act of providing that any procedure, process, equipment, material, activity or system performs as expected under given set of conditions and also give the required accuracy, precision, sensitivity, ruggedness, etc.

The various validation parameters are:

- Accuracy
- Precision (repeatability and reproducibility),
- Linearity and range,
- Limit of detection (LOD)
- Limit of quantification (LOQ)
- Selectivity / specifity,
- Robustness / ruggedness
- Stability and system suitability studies.

**1. Accuracy:**

The accuracy of an analytical method may be defined as the closeness of the test results obtained by the method to the true value. It is the measure of the exactness of the analytical method developed. Accuracy may often express as percent recovery by the assay of a known amount of analyte added.

Accuracy may be determined by applying the method to samples or mixtures of excipients to which known amount of analyte have been added both above and below the normal levels expected in the samples. Accuracy is then calculated from the test results as the percentage of the analyte recovered by the assay. Dosage form assays commonly provide accuracy within 3-5% of the true value. The ICH recommend that accuracy should be assessed using a minimum of nine determinations over a minimum of three concentration levels, covering the specified range ( i.e. three concentration and three replicated of each concentration).

Percentage recovery = T - A X 100 / S

Where T = Total amount of drug estimated

A= Amount contributed by formulation

S = Amount of pure drug added.

**2. PRECISION:**

Precision of analytical method is expressed as SD and RSD of series of measurement by replicate estimation of drug. Repeatability involves analysis of replicates by the analyst using the same equipment and method and conducting the precision study over short period of time while reproducibility involves precision study at

- Different Occasions,
- Different Laboratories,
- Different Batch of Reagent,
- Different Analysts,
- Different Equipments.

The RSD values are important for showing degree of variation expected when the analytical procedure is repeated several time in a standard situation. (RSD below 1% for bulk drugs, RSD below 2% for assays in finished product).

**3. LINEARITY AND RANGE:**

The linearity of an analytical method is its ability to elicit test results that are directly proportional to the analyte concentration in sample within a given range. Linearity usually Expressed in terms of the variance around the slope of regression line calculated according to an established mathematical relationship from test results obtained by the analysis of samples

With varying concentrations of analyte.The working sample concentration and sample tested For accuracy should be in the linear range. The claim that the method is linear to be justified with additional mention of zero intercept by processing data by linear least square regression Declaring the regression co-efficient and b of the linear equation y = ax+ b together with the Correlation coefficient of determination 'r'. For the method to be linear the 'r' value should be close to 1.

**4. LIMIT OF DETECTION AND LIMIT OF QUANTITATION:-**

**LIMIT OF DETECTION:**

The limit of detection is the parameter of limit tests. It is the lowest level of analyte that can be detected, but not necessarily determined in a quantitative fashion, using a specific method under the required experimental conditions. The determination of the limit of detection of instrumental procedures is carried out by determining the signal-to-noise ratio by comparing test results from the samples with known concentration of analyte with those of blank samples and establishing the minimum level at which the analyte can be reliably detected. A signal-to-noise ratio of 2:1 or 3:1 is generally accepted.

For spectroscopic techniques or other methods that rely upon a calibration curve for quantitative measurements, the IUPAC approach employs the standard deviation of the intercept ($S_a$) which may be related to LOD and the slope of the calibration curve, b, by

$$\mathbf{LOD = 3\ S_a / b}$$

**LIMIT OF QUANTITATION:**

The limit of quantitation is the lowest concentration of analyte in a sample that may be determined with acceptable accuracy and precision when the required procedure is applied.

It is measured by analyzing samples containing known quantities of the analyte and determining the lowest level at which acceptable degrees of accuracy and precision are attainable where the final assessment is based on an instrumental reading, the magnitude of background response by analyzing a number of blank samples and calculating the standard deviation of this response. The standard deviation multiplied by a factor (usually 10) provides an estimate of the limit of quantitation. In many cases, the limit of quantitation is approximately twice the limit of detection.

**5. SELECTIVITY AND SPECIFICITY:**

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix. If any analytical procedure is able to separate and resolve the various components of a mixture and detect the analyte qualitatively the method is called selective. On the other hand, if the method determines or measures quantitatively the component of interest in the sample matrix without separation, it said to be specific.

Selectivity may be expressed in terms of the assay results obtained when the procedure is applied to the analyte in the presence of expected levels of other components, compared the results obtained when the procedure is applied to the analyte in the presence of expected levels of other components, compared to the results obtained on the same analyte without added substances. When the other components are all known and available, selectivity may be determined by comparing the test results obtained on the analyte with and without the addition of the potentially interfering materials. When such components are either unidentified or unavailable, a measure of selectivity can often be obtained by determining the recovery of a standard addition of pure analyte to a material containing a constant level of the other components.

**6. ROBUSTNESS AND RUGGEDNESS:**

**ROBUSTNESS:**

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. The determination of robustness requires that methods characteristics are assessed when one or more operating parameter varied.

Ruggedness:

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different laboratories, different analysts, using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The testing of ruggedness is normally suggested when the method is to be used in more than one laboratory. Ruggedness is normally expressed as the lack of the influence on the test results of operational and environmental variables of the analytical method. For the determination of ruggedness, the degree of reproducibility of test result is determined as function of the assay variable. This reproducibility may be compared to the precision of the assay under normal condition to obtain a measure of the ruggedness of the analytical method.

**7. STABILITY AND SYSTEM SUITABILITY TESTS:**

Stability of the sample, standard and reagents is required for a reasonable time to generate reproducible and reliable results. For example, 24 hour stability is desired for solutions and reagents that need to be prepared for each analysis.

System suitability test provide the added assurance that on a specific occasion the method is giving, accurate and precise results. System suitability test are run every time a method is used either before or during analysis. The results of each system suitability test are compared with defined acceptance criteria and if they pass, the method is deemed satisfactory on that occasion. The nature of the test and the acceptance criteria will be based upon data generated during method development optimization and validation experiments.

## 2. REVIEW OF LITERATURE

- **Selvadurai Muralidharan *et al*[24];** reported a method development and validation for the quantitative determination of Dexibuprofen (DI) in pharmaceutical dosage form. The method was carried out by reversed phase HPLC technique on a RP-18 column with a mobile phase composed of acetonitrile and 0.5 % triethylamine (pH 7.5 adjusted with orthophosphoric acid (30:70,v/v)). UV method was performed with the $\lambda_{max}$ at 222.0 nm. Validation parameters such as linearity, precision, accuracy, and specificity were determined.

- **Wan Wen Qing *et al*[25];** reported a establish a method for content determination of dexibuprofen in the gel preparation by HPLC. Hypersil ODS2 column was used with acetonitrile: water (pH 3.0) 58:42 as mobile phase detecting wavelength as 263 nm flow rate as 1.0 ml min and temperature as 35ºC. The Dexibuprofen showed a good linear correlation with a range of 25.1-251.0 µg/ml, r = 0.9999. The average recovery was 101.6%.

- **A. Thenmozhi *et al*[26];** reported a method development and validation for Dexibuprofen in tablet dosage form by RP-HPLC. The method was carried out on Waters Symmetry C18 column in isocratic mode, with mobile phase consisting of Acetonitrile and water in the ratio of 55: 45 % (v/v), adjusted to pH 2.5 with orthophosphoric acid. The mobile phase was pumped at a rate of 1.5mL/min and detection was carried out at 214nm and linearity was found to be in the range of 40 to 160µg/ml. The correlation coefficient was found to be 0.9991. Validation of the proposed method has also been done.

- **P. Balan *et al*[27];** reported a method development and validation for the determination of Dexibuprofen and Paracetamol in combined tablet dosage form by RP-HPLC. The separation was carried out by using a mobile phase consisting of Acetonitrile: water in ratio of 50: 50 pH 7.8 adjusted with Triethylamine. The column used was C18, 250 × 4.5 mm with flow rate of 1.0 ml / min using UV detection at 230 nm. The described method was linear over a concentration range of 2-10 µg/ml (r2 =0.999485 and0.999433) and the retention times of Dexibuprofen and Paracetamol were found to be 1.763 and 2.463 min respectively. The method was validated as per ICH guidelines.

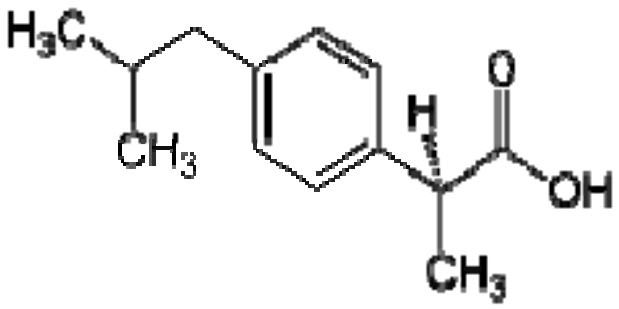
- **Selvadurai Muralidharan *et al*[28];** reported a method development and validation for analysis of Dexibuprofen in tablet dosage form by HPTLC. The method uses aluminium foil HPTLC plates coated with silica gel 60F 254 as stationary phase and hexane: ethyl acetate: glacial acetic acid 7.5: 2.5: 0.5 (v/v) as mobile phase. Densitometric analysis of dexibuprofen and the internal standard (aceclofenac) was performed in reflectance mode at 217 nm. The system was found to give compact bands for Dexibuprofen (RF 0.50). Linearity was found to be in the range of 50-300 ng per band (r2 = 0.9902).

## 3. DRUG PROFILE

### DEXIBUPROFEN:

- Not official in any pharmacopeia
- Dexibuprofen is a Non -Steroidal Anti Inflammatory and Analgesic effect. It is the active dextrorotatory enantiomer of ibuprofen.

### STRUCTURAL FORMULA[29]:




### CHEMICAL NAME:

Chemically it is (2S)-2-[4-(2-methyl propyl) phenyl] propanoic acid(1,2,3)

### MOLECULAR FORMULA:

$C_{13}H_{18}O_2$

### MOLECULAR WEIGHT:

206.28gm/mol

### DESCRIPTION:

A white colour powder.

### SOLUBILITY:

Readily soluble in organic solvent such as ethanol and acetone and slightly soluble in water.

### TABLET FORMULATION:

Dexibuprofen 300 mg.

**THERAPEUTIC CATEGORY:**

Non steroidal anti inflammatory drug.

**PHARMACOKINETICS[30-33]:**

Dexibuprofen is absorbed primarily from the small intestine. After metabolic transformation in the liver (hydroxylation, carboxylation), the pharmacologically inactive metabolites are completely excreted, mainly by the kidneys (90%), but also in the bile. The elimination half-life is 1.8 - 3.5 hours; the plasma protein binding is about 99 %. Maximum plasma levels are reached about 2 hours after oral administration.

**INDICATION :**

- Pain and inflammation caused by osteoarthritis.
- Acute symptomatic treatment of pain during menstrual bleeding (primary dysmenorrhoea).
- Mild to moderate pain, such as pain in the muscles and joints and toothaches.

**CONTRA INDICATION:**

- Hypersensitivity to aspirin or other non-steroidal anti-inflammatory drugs.
- Active or suspected gastrointestinal ulcer .
- Gastrointestinal bleeding or other active bleedings or bleeding disorders.
- Active Crohn's disease or active ulcerative colitis.
- Severe renal dysfunction ,severely impaired hepatic function.
- Haemorrhagic diathesis and other coagulation disorders.

**PRECAUTIONS:**

- History of bronchial asthma
- Renal or hepatic disorder
- Bleeding disorders
- Cardio vascular disease.

**ADVERSE DRUG REACTION :**

GI bleeding, heartburn, epigastric pain, dyspepsia, peptic ulcer, nausea, vomiting, diarrhoea, jaundice, hepatitis, rash, thrombocytopaenia, visual disturbances, depression, fatigue, headache, dizziness, vertigo.

**DRUG INTERACTIONS:**

- **Anticoagulants:** The effects of anticoagulants on bleeding time can be potentiated by NSAIDs. If concomitant treatment cannot be avoided blood coagulation tests (INR, bleeding time) should be performed during the initiation of Dexibuprofen treatment and the dosage of the anticoagulant should be adjusted if necessary .
- **Methotrexate used at doses of 15 mg/week or more:** If NSAIDs and methotrexate are given within 24 hours of each other plasma levels of methotrexate may increase, via a reduction in its renal clearance thus increasing the potential for methotrexate toxicity. Therefore, in patients receiving high-dose treatment with methotrexate, the concomitant use of Dexibuprofen is not recommended.
- **Lithium:** NSAIDs can increase the plasma levels of lithium, by reducing its renal clearance. The combination is not recommended. Frequent lithium monitoring should be performed. The possibility of reducing the dose of lithium should be considered other NSAIDs and salicylates ( acetyl salicylic acid at doses above those used for anti-thrombotic treatment, approximately 100 mg/day ) The concomitant use with other NSAIDs should be avoided, since simultaneous administration of different NSAIDs can increase the risk of gastrointestinal ulceration and haemorrhage.

## 4. AIM AND OBJECTIVE

- Analytical methods by using sophisticated instruments such as HPLC and HPTLC are having the vital role in the modern pharmaceutical analysis. Application of these instruments for qualitative and quantitative estimation of marketed formulation.
- In this research work we applied two instruments namely HPLC and HPTLC for estimation of Dexibuprofen.
- The literature review reveals that one HPTLC method and few HPLC methods in Pharmaceutical formulation for the quantification of dexibuprofen.
- The aim of the present work was to develop and validate a simple, fast, and reliable RP-HPLC and HPTLC method for the determination of dexibuprofen in tablet dosage form.
- Conformation of the applicability of the developed method was validated according to the International Conference on Harmonisation to determination of dexibuprofen in pharmaceutical dosage forms.

# 5. PLAN OF WORK

**Development of Validated RP-HPLC and HPTLC Method for Estimation of Dexibuprofen in pharmaceutical dosage forms.**

The plan of the proposed work includes the following steps.

- Selection of the drug.
- Collection of all available information and different analytical procedures related to the drug.
- To undertake solubility study for the Dexibuprofen.
- Selection of suitable stationary phase and mobile phases.
- Selection of detection wave length.
- Optimization of chromatographic parameters.
- Preparation of calibration curve.
- Estimation of Dexibuprofen formulation.
- To validate analytical method developed as per the ICH guidelines.

# 6. LIST OF INSTRUMENTS USED

| S.NO | INSTRUMENTS |
|---|---|
| 1. | SHIMADZU HPLC with UV detector |
| 2. | HYPERSIL BDS C18 column (150 × 4.6mm, 5µ) |
| 3. | CAMAG HPTLC instrument |
| 4. | CAMAG TLC SCANNER 3 |
| 5. | CAMAG LINNOMATE V AUTOMAIC SAMPLE APPLICATOR |
| 6. | Twin-trough chamber (10 × 10 cm) |
| 7. | Ultra sonicator |
| 8. | Electronic balance (2mg-200gm) (Sartorius) |
| 9. | pH Analyser |

# LIST OF CHEMICALS AND SOLVENTS

| S.NO | CHEMICALS AND SOLVENTS | MANUFACTURER |
|---|---|---|
| 1. | DEXIBUPROFEN working standard | USP standards |
| 2. | Acetonitrile (HPLC grade) | Rankem |
| 3. | Methanol (HPLC grade) | Fischer scientific |
| 4. | Tri ethyl amine | Merck |
| 5. | Toluene | Fischer scientific |
| 6. | Chloroform | Merck |
| 7. | Ethanol | Merck |
| 8. | Acetone | Merck |
| 9. | Ethyl acetate | Fischer scientific |
| 10. | Glacial acetic acid | Merck |
| 11. | Milli-Q/HPLC water | Merck |

## 7. METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF DEXIBUPROFEN TABLET DOSAGE FORM BY RP-HPLC

**1. Selection of Column:**

Experiments with different $C_{18}$ columns were conducted to achieve best separation. $C_{18}$ columns such as Hypersil BDS $C_{18,}$ was tried. After reviewing the results it was found that the peak shape, retention time, resolution, tailing factor, and column efficiency are good with Hypersil BDS $C_{18}$ (150 x4.6mm,5.0µm) column was selected.

**2. SELECTION OF DETECTION WAVELENGTH:**

The sensitivity of the HPLC method that uses UV detector depends upon the proper selection of wavelength. An ideal wavelength which gives good response for the drug to be detected is to be selected from the UV spectrum obtained for the drug, 264 nm was selected as the wavelength for this method.

**INITIAL CHROMATOGRAPHIC CONDITIONS:**

| | | |
|---|---|---|
| Stationary phase | : | Hypersil BDS C18, (150 × 4.6 mm, 5µm particle size) |
| Flow rate | : | 1.0 ml/minutes |
| Column temperature | : | Ambient |
| Wavelength selected | : | 264 nm |

**3. SELECTION OF MOBILE PHASE:**

Experiments with different mobile phases were tried in different ratio for the selection of mobile phase. So we tried with a mixture of solvent like Acetonitrile: water for getting a suitable symmetrical peak. After repeated trials this mixture of solvents Acetonitrile: water solvent gives a good symmetrical peak. So the above mobile phase was used as a solvent of choice for the drug.

**5. EFFECT OF RATIO OF MOBILEPHASE:**

The mobile phase of Acetonitrile : water in various of 50:50, 55:45, 60:40, were tried and the chromatogram were recorded at 264 nm at the ratio of 60:40%v /v was selected as the ideal ratio for the estimation of dexibuprofen. But the peak shape was not good so we add the

peak modifiers such as 1 ml of glacial acetic acid and 0.2 ml of triethylamine and adjust the pH to 5.0± 0.05 to get a sharp symmetrical peak with good retention time.

**6. EFFECT OF FLOW RATE:**

Keeping the mobile phase ratio of Acetonitrile: water (60:40%v/v) the chromatogram was recorded at flow rate like 1.0ml/min. In this flow rate the peak was sharp with good resolution and it was kept constant for the analysis.

**TRIAL: 1**

**CHROMATOGRAPHIC CONDITION:**

Stationary phase : Hypersil BDS C18, (150 × 4.6 mm, 5µm particle size)

Flow rate : 1.0 ml/minutes

Column temperature : Ambient

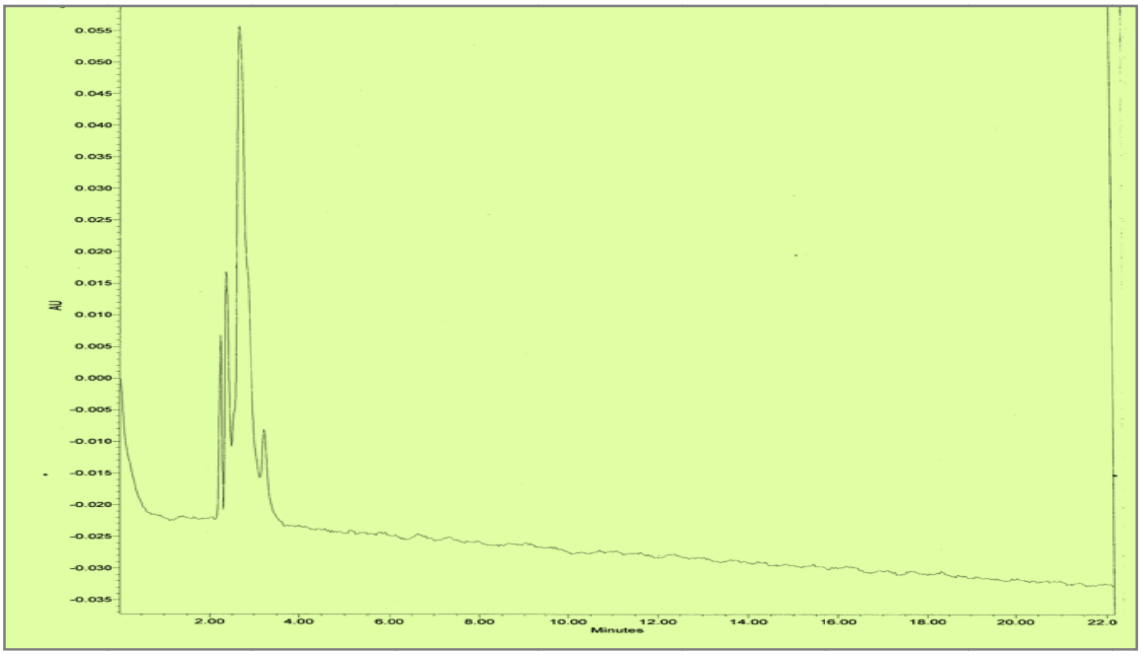
Wavelength selected : 264 nm

Injection volume : 10µl

Run time : 10 minutes

Mobile phase : Acetonitrile: water (50: 50%v/v)

**Fig No: 2 Chromatogram Showing Trial No: 1**

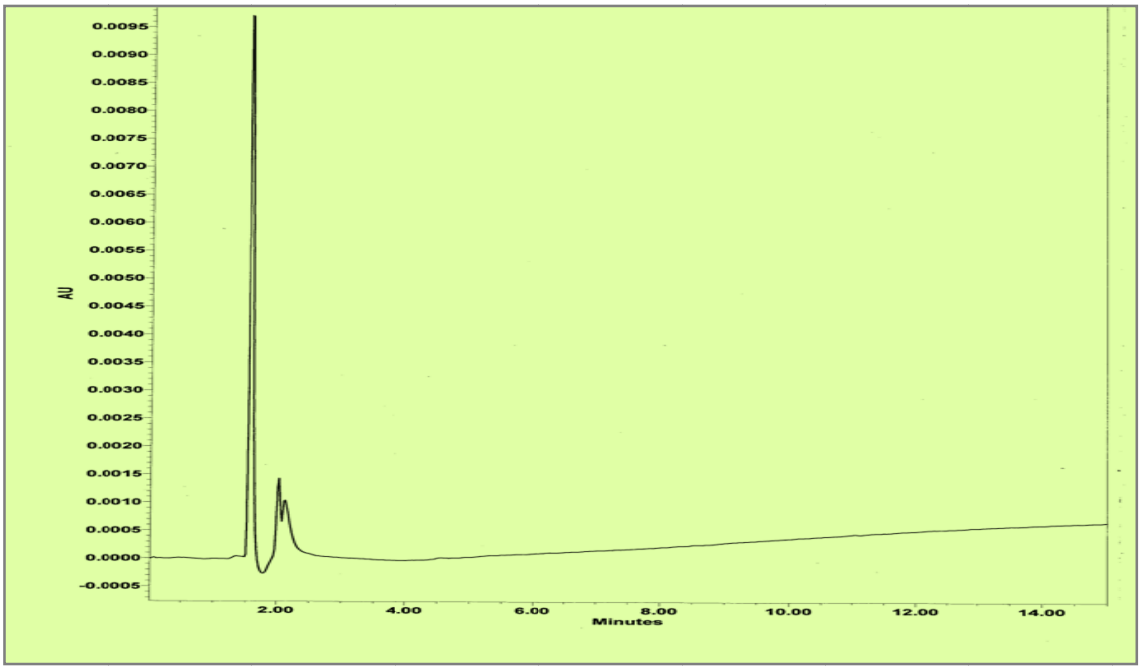


**TRIAL: 2**

**CHROMATOGRAPHIC CONDITIONS:**

| | | |
|---|---|---|
| Stationary phase | : | Hypersil BDS C18, (150 × 4.6 mm, 5µm particle size) |
| Flow rate | : | 1.0 ml/minutes |
| Column temperature | : | Ambient |
| Wavelength selected | : | 264 nm |
| Injection volume | : | 10µl |
| Run time | : | 10 minutes |
| Mobile phase | : | Methanol: water (55:45% V/V) |

**Fig No: 3 Chromatogram Showing Trial No: 2**



**TRIAL: 3**

**CHROMATOGRAPHIC CONDITION:**

Stationary phase : Hypersil BDS C18, (150 × 4.6 mm, 5µm particle size)

Flow rate : 1.0 ml/minutes

Column temperature : Ambient

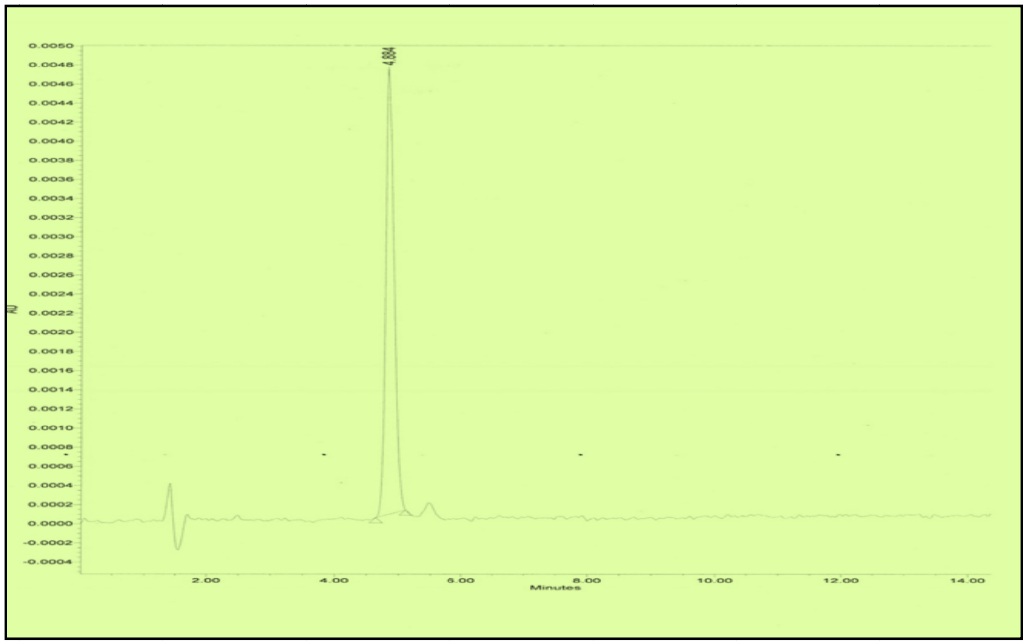
Wavelength selected : 264 nm

Injection volume : 10µl

Run time : 10 minutes

Mobile phase : Acetonitrile: water (60: 40%v/v) add 0.1 ml of triethylamine.

**Fig No: 4 Chromatogram Showing Trial No: 3**



**FIXED CHROMATOGRAPHIC CONDITION:**

Stationary phase : Hypersil BDS C18 (150 × 4.6 mm, 5µm particle size)

Flow rate : 1.0 ml/minutes

Column temperature : Ambient

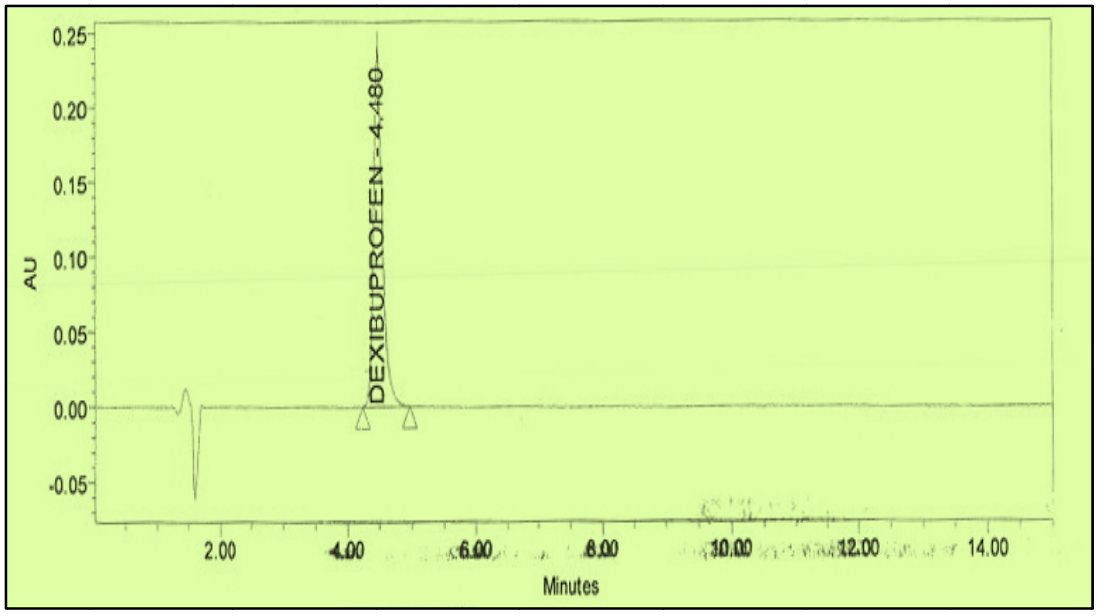
Wavelength selected : 264 nm

Injection volume : 10µl

Run time : 10 minutes

Mobile phase : Acetonitrile: Water (60:40%v/v) 1 ml of glacial acetic acid and 0.2 ml triethylamine. Adjust the pH to 5.0 with glacial acetiacid

**Fig No: 5 Chromatogram Showing Dexibuprofen**



**PREPARATION OF MOBILE PHASE:**

Mix 600 ml of acetonitrile, 400ml of water, 1 ml of glacial acetic acid and 0.2 ml of triethylamine. Adjust the pH to 5.0± 0.05 with triethylamine or glacial acetic acid. Filter the solution through 0.45µm membrane filter.

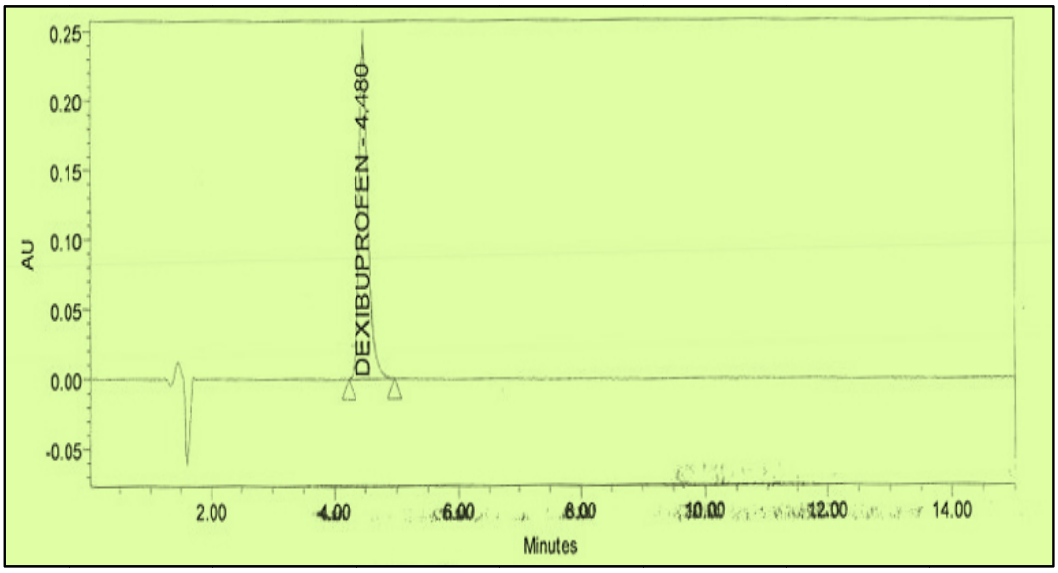
**PREPARATION OF DILUENT:**

Prepare a mixture of 600 ml of acetonitrile and 400 ml of water.

**STANDARD PREPARATION:**

weigh accurately and transfer about 50 mg of Dexibuprofen working reference standard into a 50 ml volumetric flask, add 30 ml of methanol sonication to dissolve the content and make up to the volume with methanol. Dilute 5 ml of the above solution to 50 ml with diluent and mix well. Chromatogram of standard solution is as shown in Fig No: 6 and assay results are tabulated and are as shown in Table No: 1.

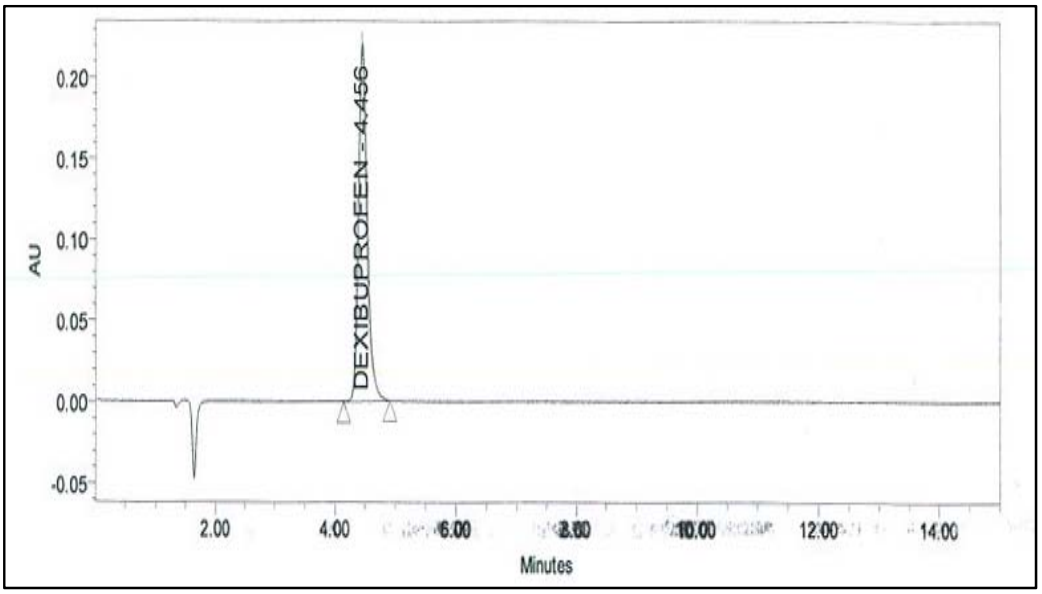
**Fig. No: 6 Chromatogram showing peaks of standard solution of Dexibuprofen**



**SAMPLE PREPARATION:**

Weigh and transfer 8 tablets into a 200 ml volumetric flask, add 10 ml of water and shake for 5 min. Add 100 ml of methanol sonication for 20 minutes with intermediate shaking, make up to the volume with methanol and mix well. Filter the solution through a 0.45µm nylon filter. Dilute 2.0 ml of filtrate to 250 ml volumetric flask and make up to volume with diluents. Chromatogram of sample solution is as shown in Fig No: 7

**Fig. No: 7 Chromatogram showing peaks of sample solution of Dexibuprofen**




| Name | Retention time (min) | Area | USP Tailing | USP Plate count |
|---|---|---|---|---|
| Dexibuprofen | 4.480 | 2447677 | 1.2 | 4885 |

**EVALUATION OF SYSTEM SUITABILITY:**

- The relative standard deviation for the area of five replicate injections of Dexibuprofen standard solution-2 should be less than 2.0.
- Tailing factor for Dexibuprofen peak in the first injection of standard solution-2 should be less than 2.0.
- Theoretical plates for Dexibuprofen peak in the first injection of standard solution-2 should be more than 2000.

**METHOD VALIDATION:**

Validation is the process of evaluating products or analytical methods. It includes testing but it is more for instance, the checking the documentation for completeness and correctness.

Validation is defined as documented evidence that a system performance as expected.

**OBJECTIVE:**

The objective of this validation study is to demonstrate that the proposed method is suitable for its intended use.

This study covers the following parameters,

1. Precision
    i. System precision
    ii. Method precision
    iii. Intermediate precision
2. Specificity
3. Forced degradation
4. Linearity
5. Accuracy
6. Solution stability
7. Filter interference study
8. Robustness

**SYSTEM SUITABILITY TESTING:**

The purpose of the system suitability test is to ensure that the complete testing system (including instrument, reagents, columns, analysts) is suitable for the intended application. System suitability tests are an integral part of liquid chromatographic methods. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done.

**EVALUATION OF SYSTEM SUITABILITY**

1. The relative standard deviation for the area of five replicate injections of dexibuprofen standard solution should be less than 2.0
2. Tailing factor dexibuprofen peak in the first injection of standard solution should be less than 2.0
3. Theoretical plates for dexibuprofen peak in the first injection of standard solution should be more than 2000 and the results are tabulated and are as shown in Table No

**1. PRECISION:**

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: Repeatability, Intermediate precision and Reproducibility.

**PROCEDURE:**

**A) SYSTEM PRECISION:**

**Preparation of standard solution (100µg/ml):**

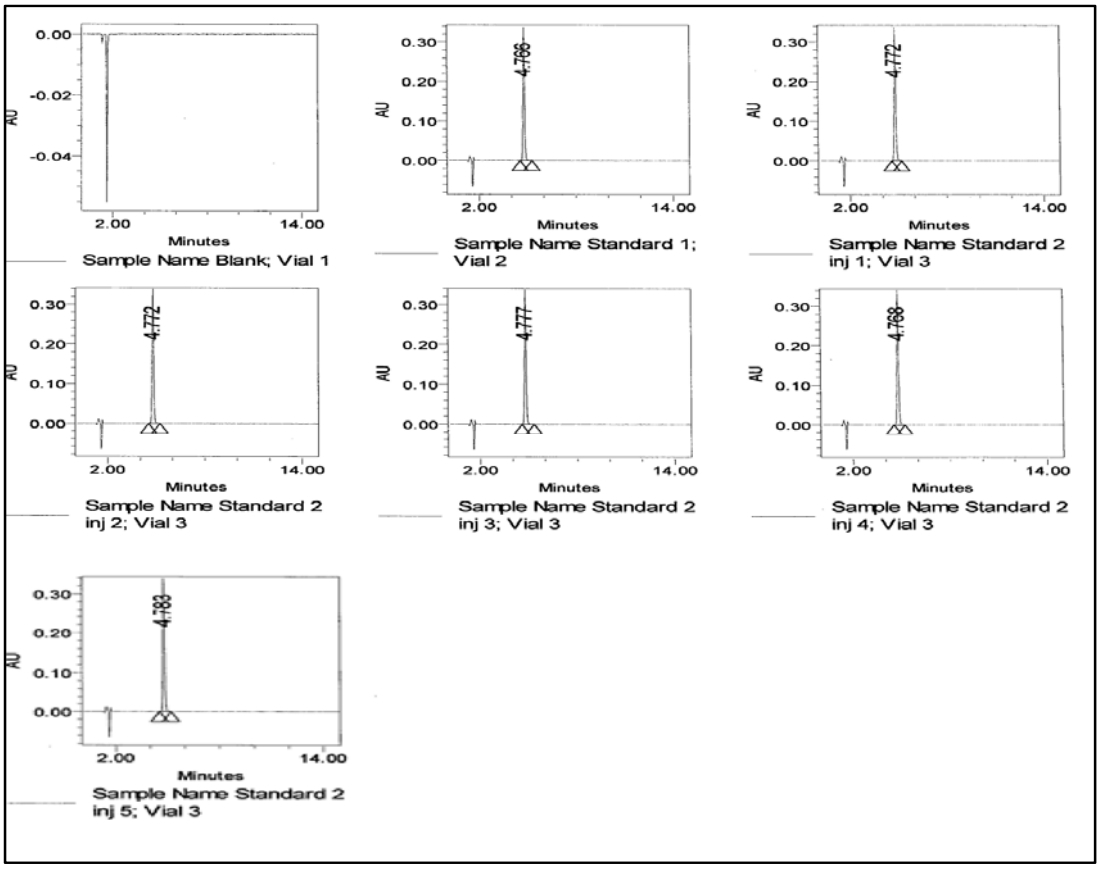
Weigh accurately and transfer about 50.0 mg of dexibuprofen standard into a 50.0 ml volumetric flask, add 30 ml of methanol sonication to dissolve the content and make up to the volume with methanol (stock solution).

Dilute 5.0 ml of the above solution to 50.0 ml with diluent and mix well. Inject 10µl of the blank solution and the standard solution of 100µg/ml for five times and calculate the %RSD for the area of five replicate injections. The chromatograms are as shown in Fig No: 8 and the results are tabulated in table – 2.

**ACCEPTANCE CRITERIA:**

Percentage relative standard deviation (%RSD) for peak areas of dexibuprofen is not more than 2.0.

**Fig No: 8 Chromatogram showing System Precision of Dexibuprofen**




**B) METHOD PRECISION:**

**Preparation of sample solution:**

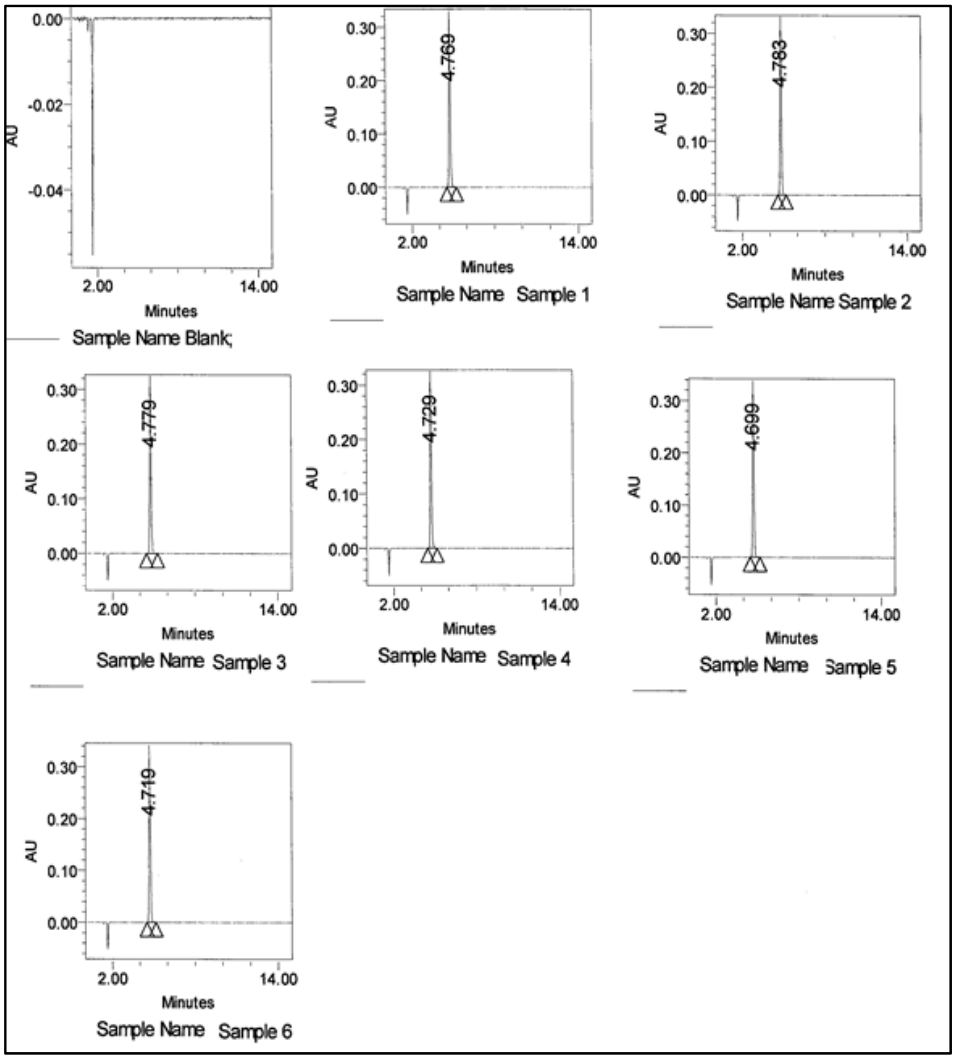
Weigh and transfer 8 tablets in to a 200.0 ml volumetric flask, add 10 ml of water and shake for 5 minutes. Add 100 ml of methanol, sonication for 20 minutes with intermediate shaking and make up to the volume with methanol. Mix well and filter the solution through a 0.45µm nylon filter. Dilute 2.0 ml of filtrate to 250.0 ml with diluent.

Inject 10µl of the blank solution and six replicate injections of sample solution for six times and calculate the %RSD for the area of six replicate injections. The chromatograms are as shown in Fig No: 9 and the results are tabulated in table – 3.

**ACCEPTANCE CRITERIA:**

Percentage relative standard deviation (%RSD) for percentage of assay is not more than 2.0.

**Fig No: 9 Chromatogram showing Method Precision of Dexibuprofen**



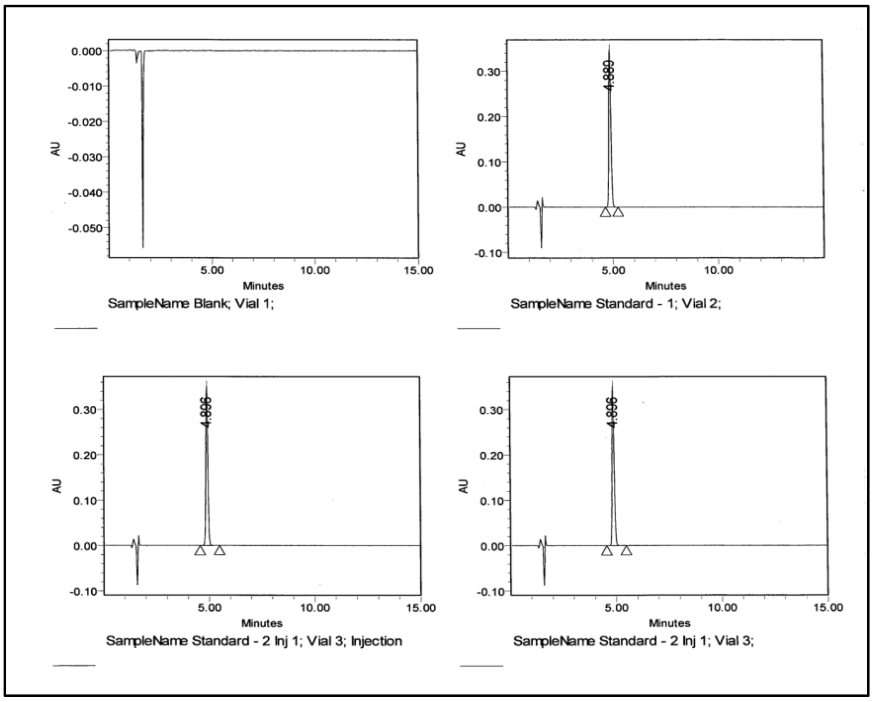

**C) INTERMEDIATE PRECISION (RUGGEDNESS):**

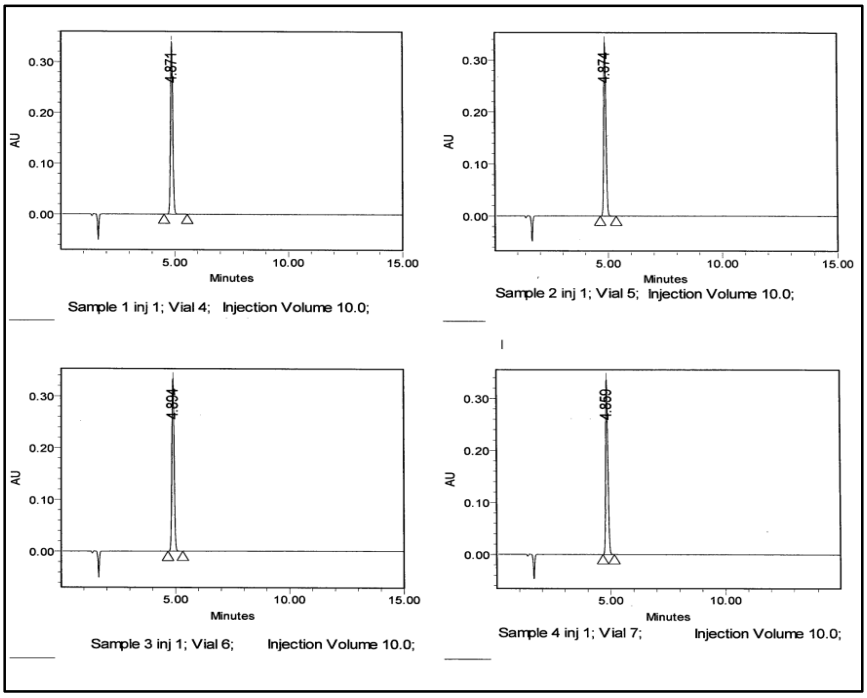
Ruggedness of the method was verified by analysing the six samples of 300 mg tablets of same batch which was used for method precision as per testing procedure. This study was performed by different analyst using different instrument and different column on different day. Calculated the percentage assay and percentage relative standard deviation (%RSD) for six assay results. Calculated overall percentage relative standard deviation (%RSD) for ruggedness and method precision results. The chromatograms are as shown in Fig No: 10. The results are tabulated in table – 4.

**ACCEPTANCE CRITERIA:**

Percentage relative standard deviation (%RSD) for assay is not more than 2.0.

**Fig No: 10 Chromatogram showing Intermediate Precision of Dexibuprofen**



**2. SPECIFICITY:**

Blank, placebo, standard, sample solution (unspiked) and sample solution spiked with known impurities at 1% level were injected into the HPLC system. There was no interference from the blank and placebo at the retention time of dexibuprofen peak.

Peak purity data reveals that dexibuprofen peak was homogenous and there were no co-eluting peaks at the retention time of dexibuprofen peak.

Calculated the percentage difference between the mean assay of unspiked and spiked sample with respect to unspiked sample. The results are tabulated in table – 5a. The peak purity data of dexibuprofen peak from standard, sample (unspiked) and spiked sample are summarized in table – 5b. The chromatograms are as shown in Fig No: 11 and 12.

**ACCEPTANCE CRITERIA:**

i. No peaks elutes at the retention time of dexibuprofen in blank and placebo.
ii. The percentage difference between the assay values of spiked and unspiked sample is not more than ± 2.0 with respect to unspiked sample.
iii. Peak purity passes.

**Fig No: 11 Chromatogram showing specificity of Dexibuprofen**

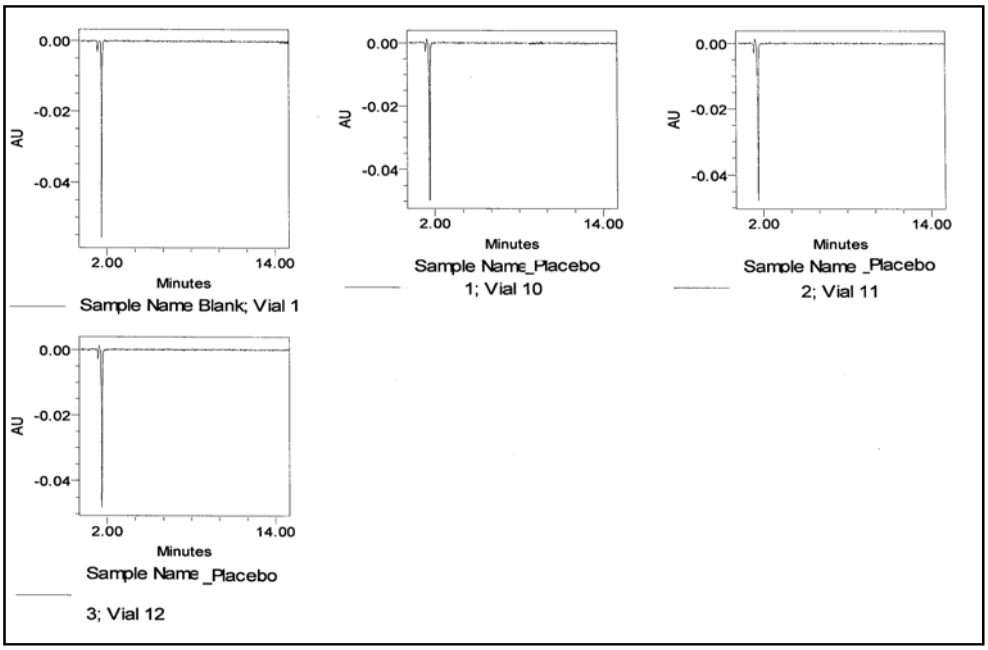
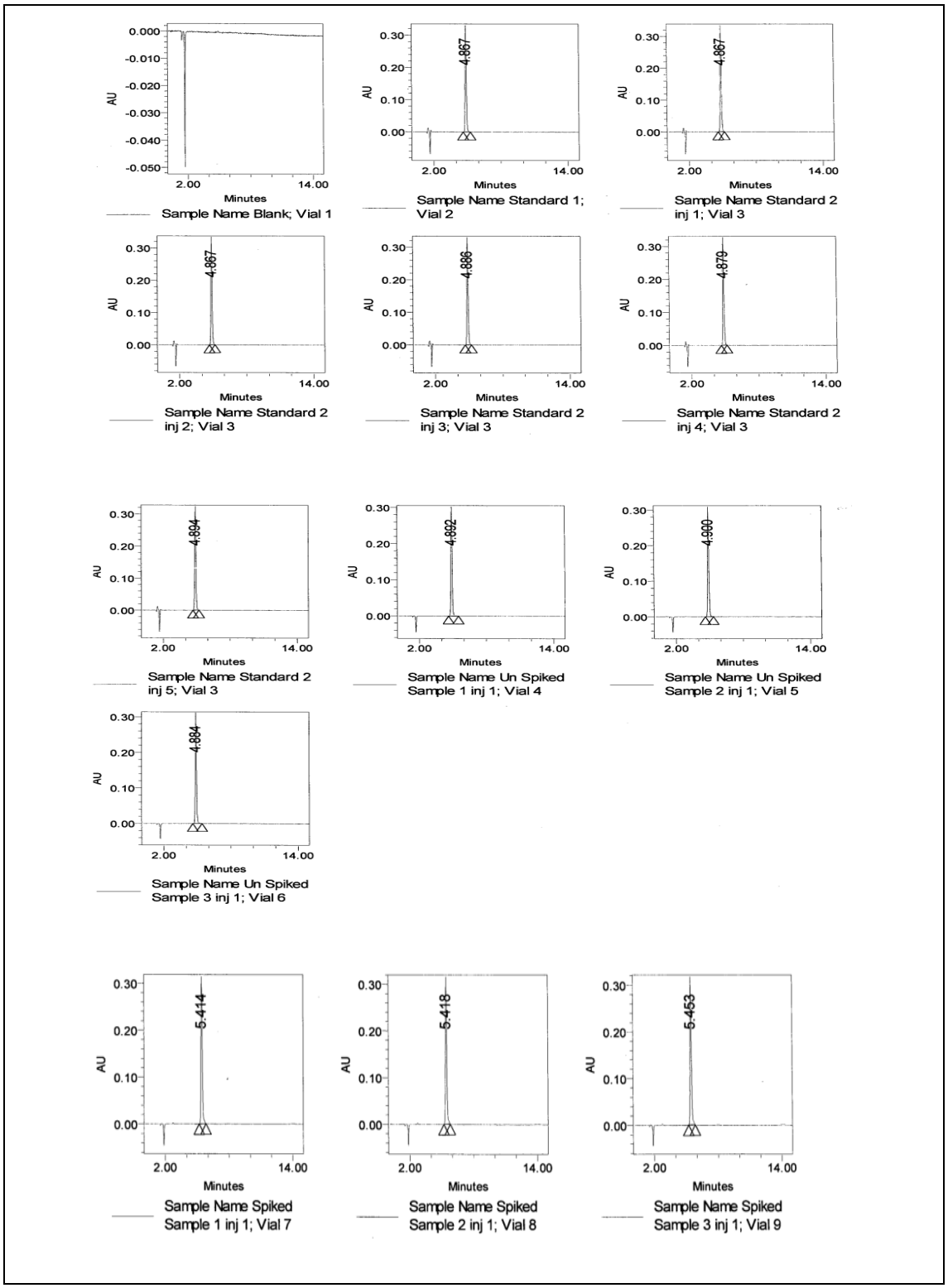


**Fig No: 12 Chromatogram showing specificity of Dexibuprofen**



**3. FORCED DEGRADATION:**

Forced degradation study was carried out by treating the sample under the following conditions.

**a) Degradation By Hydrochloric Acid (Acid Treated Sample):**

Sample was treated with 10 ml of 5N Hydrochloric acid and kept on bench top for 12 hours. Treated sample solution was analysed as per the testing procedure.

**b) Degradation By Sodium Hydroxide (Base Treated Sample):**

Sample was treated with 10 ml of 5N sodium hydroxide and kept on bench top for 12 hours. Treated sample solution was analysed as per the testing procedure.

**c) Degradation By Hydrogen Peroxide (Peroxide Treated Sample):**

Sample was treated with 10 ml of 30% solution of hydrogen peroxide and kept on bench top for 12 hours. Treated sample solution was analysed as per the testing procedure.

**d) Degradation By Thermal (Heat Treated Sample):**

Sample was kept in an oven at 105ºc for about 24 hours. Treated sample was analysed as per the testing procedure.

**e) Degradation By Photo Light [Controlled Condition (Wrapped In Aluminium Foil)]:**

Sample was exposed to light of 1.2 million lux hours in protected condition. Treated sample was analysed as per the testing procedure.

**f) Degradation By Photo Light [Uncontrolled Condition]:**

Sample was exposed to light of 1.2 million lux hours. Treated sample was analysed as per the testing procedure.

The results of forced degradation studies are summarized in table – 6.

**ACCEPTANCE CRITERIA:**

i. Peak purity for main peak passes.
ii. Degradation is not more than 30% in each condition.

**4. LINEARITY & RANGE:**

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

**PROCEDURE:**

**Preparation of standard stock solution:**

Weigh accurately and transfer about 50.0 mg of dexibuprofen standard into a 50.0 ml volumetric flask, add 30 ml of methanol sonication to dissolve the content and make up to the volume with methanol.

**Preparation of (20, 40, 60, 80, and 100 µg/ml) sample solutions:**

From the above stock solution pipette out 1.0, 2.0, 3.0, 4.0, 5.0 ml respectively into individual 50ml of volumetric flasks and dilute up to the mark with diluent to prepare 20, 40, 60, 80, 100 µg/ml of sample solutions respectively.

Inject 10µl of blank solution and each linearity level standard solutions into the chromatographic system and measure the peak area.

The linearity of dexibuprofen was performed in the range of 20µg/ml to 100µg/ml (20% to 100% of working concentration). A graph was plotted with concentration (µg/ml) on x- axis and peak area on y- axis. Slope, y- intercept, correlation coefficient (r- value) were determined (Fig No: 14).

The chromatograms are as shown in Fig No: 13. The results are tabulated in table – 7.

**ACCEPTANCE CRITERIA:**

The correlation coefficient (r) value is not less than 0.99.

**Fig No: 13 Chromatogram showing Linearity of Dexibuprofen**

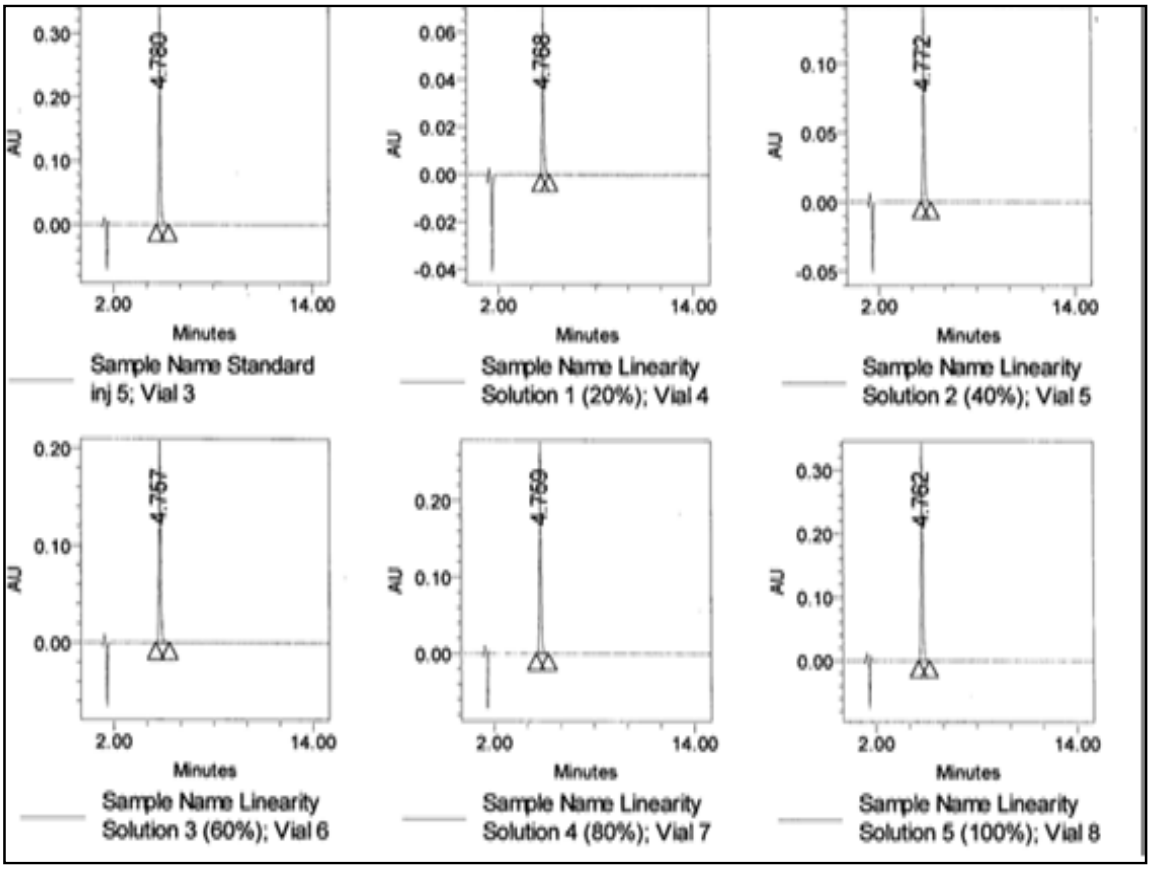
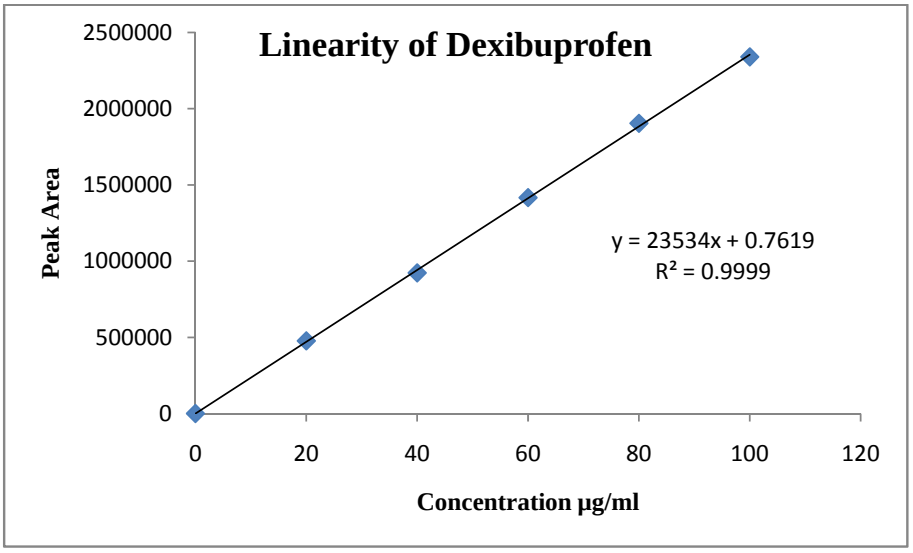



**Fig: 14 Calibration curve for Dexibuprofen**



**5. ACCURACY:**

The accuracy of an analytical method may be defined as the closeness of the test results obtained by the method to the true value. It is the measure of the exactness of the analytical method developed. Accuracy may often express as percent recovery by the assay of a known amount of analyte added.

The accuracy was demonstrated by preparing recovery sample. Known amount of dexibuprofen spiked with placebo at about 80%, 100% and 120% of working concentration in triplicate and analysed. Prepared each level in the triplicates and the average value taken to calculate the recovery. The percentage recovery was calculated from the amount found and actual amount added.

The chromatograms are as shown in Fig No: 15-16.1. The results are tabulated in table – 8.

**ACCEPTANCE CRITERIA:**

Percentage recovery at each level is in between 98.0 to 102.0

Percentage relative standard deviation (%RSD) is not more than 2.0 at each level.

**Fig No: 15 Chromatogram showing 80 %Accuracy of Dexibuprofen**

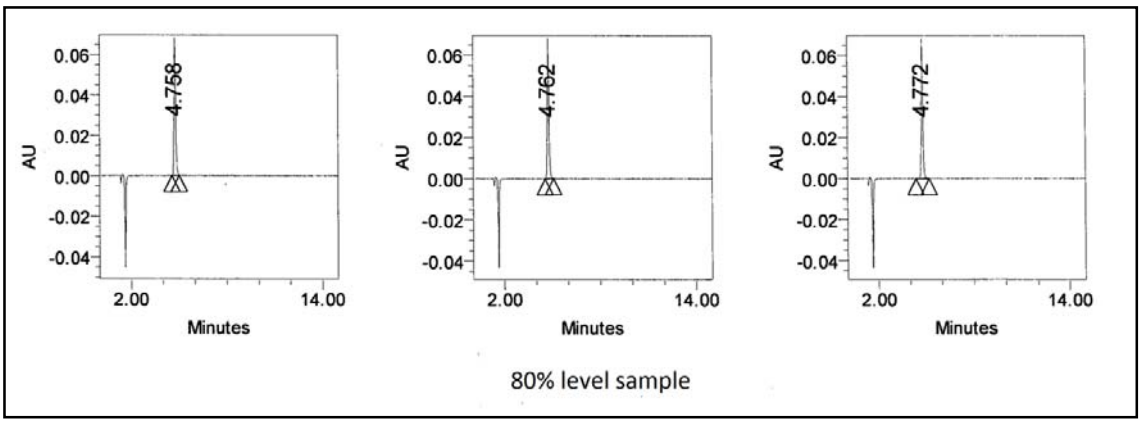


**Fig No: 16.1 Chromatogram showing 100% Acurracy of Dexibuprofen**

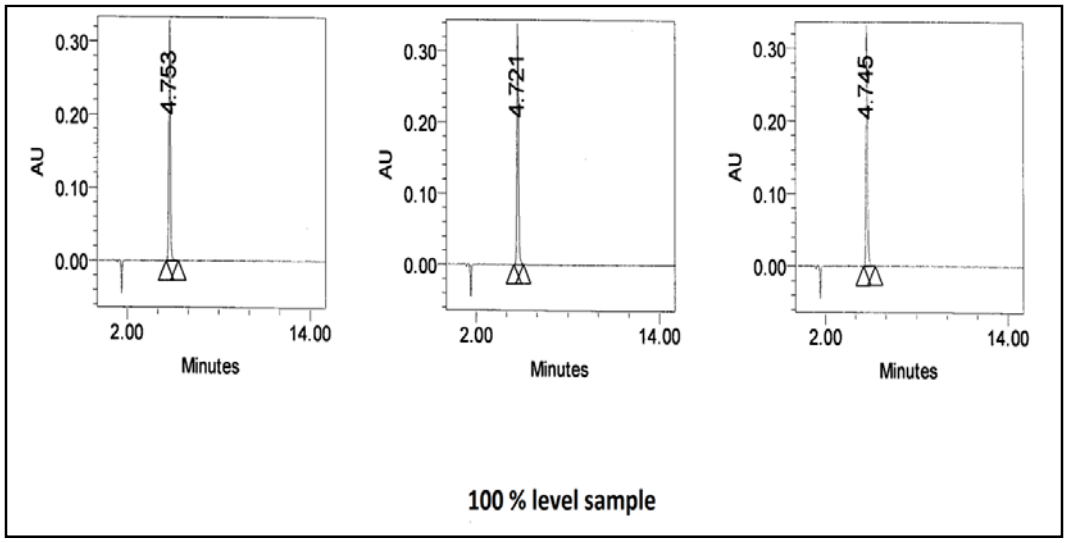
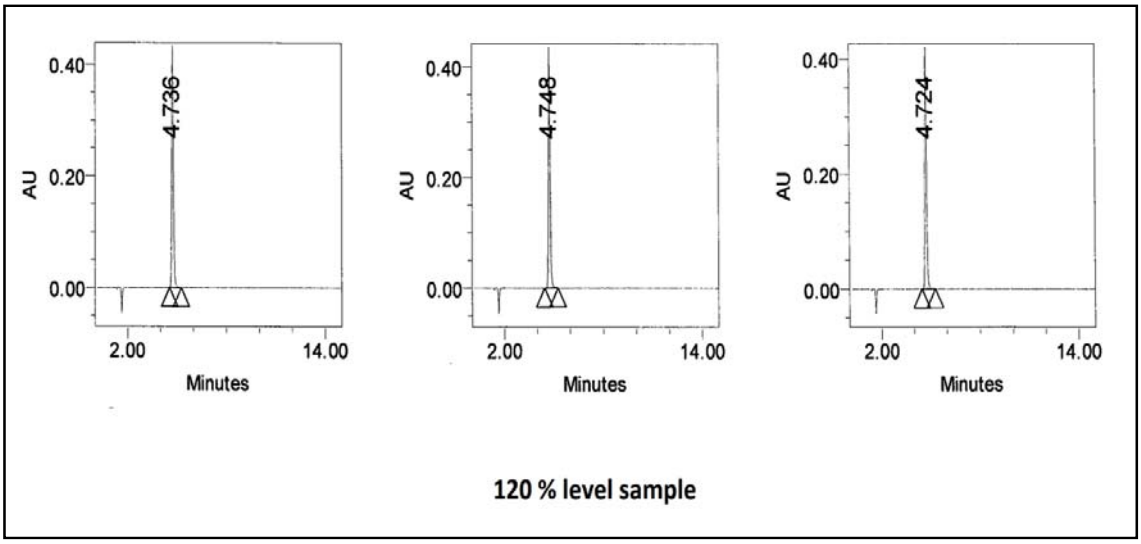



**Fig No: 16.2 Chromatogram showing 120% Acurracy of Dexibuprofen**



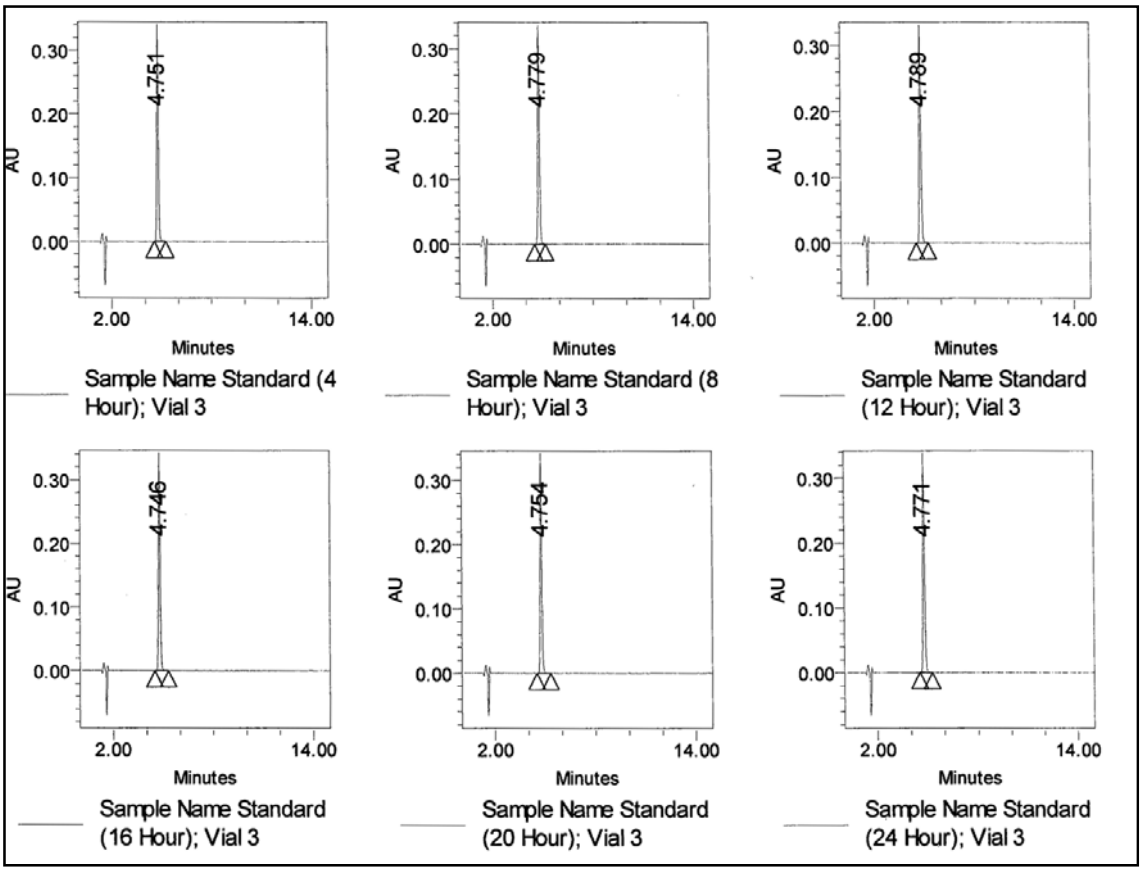
**6. SOLUTION STABILITY:**

Stability of analytical solution was verified by analysing the standard solution and sample solution of 300 mg tablets initially and also at different time intervals (4, 8, 12,16,20,24 hrs.) by storing in sample compartment of HPLC instrument at 25ºC (ambient). Calculated the cumulative percentage relative standard deviation (%RSD) for peak areas of dexibuprofen in standard and sample. The chromatograms are as shown in Fig No: 17. The results are tabulated in table – 9.

**ACCEPTANCE CRITERIA:**

Cumulative percentage relative standard deviation (% RSD) is not more than 2.0 for peaks areas of standard and sample.

**Fig No: 17 Chromatogram showing Solution stability of Dexibuprofen**



**7. FILTER INTERFERENCE STUDY:**

Filter interference study was performed on sample solution of 300 mg tablets. Prepared sample as per testing procedure filtered the solution through 0.45µ nylon filter, analysed the sample and the result compared with centrifuged sample result.

The results are tabulated in table – 10.

**ACCEPTANCE CRITERIA:**

The percentage difference between centrifuged and filtered sample results is not more than ± 2.0 with respect to centrifuged sample results.

**8. ROBUSTNESS:**

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

Robustness of the method verified by deliberately varying the following instrumental conditions.

a) By changing the flow rate by ± 0.1ml (0.9ml, and 1.1ml) variation to normal flow rate of 1.0 ml.
b) By changing the wavelength by ± 2 nm. (218nm and 220nm) variation from normal wavelength of 220nm.
c) By changing the mobile phase pH by ± 0.2 units (4.8 and 5.2) variation from normal mobile phase pH 5.0.

**Preparation of standard solution (100µg/ml):**

Weigh accurately and transfer about 50.0 mg of dexibuprofen standard into a 50.0 ml volumetric flask, add 30 ml of methanol sonication to dissolve the content and make up to the volume with methanol. Dilute 5.0 ml of the above solution to 50.0 ml with diluent and mix well.

Inject 10µl of the blank solution and the standard solution of 100 µg/ml for triplicate and analysed using varied flow rates (0.9ml, and 1.1 ml), wavelength (218nm and 222nm),

mobile phase pH (4.8 and 5.2) and calculate the %RSD for the area of triplicate injections and the results are tabulated in table – 11.

**ACCEPTANCE CRITERIA:**

Overall percentage relative standard deviation (%RSD) for assay of each change condition and the results is not more than ± 2.0.

**METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF DEXIBUPROFEN IN PHARMACEUTICAL DOSAGE FORM BY HPTLC**

**METHOD DEVELOPMENT:**

**1. Selection of Mobile Phase:**

The selection of mobile phase was done by trial and error method in which several mobile phases were tried. The best mobile phase was selected based on the $R_f$ value of the drugs. The mobile phase tried were listed below,

1. Chloroform : Ethanol (8: 2% v/v)
2. Toluene: Ethyl Acetate: Methanol: Acetic Acid (7: 1.5: 1:0.5%v/v/v/v)
3. Acetone: Benzene: Ethyl acetate: Glacial acetic acid(6: 1: 2: 1%v/v/v/v)
4. Toluene: Ethyl acetate: Glacial acetic acid (6 :3 :1% v/v/v)

**2. Fixed Mobile Phase:**

For Dexibuprofen Toluene: Ethyl acetate: Glacial acetic acid (6: 3: 1% v/v/v) was selected as mobile Phase.

**3. Activation of Pre-Coated Plates:**

Activated by placing in oven at110-120ºc for 30 minutes after sample spotting

**4. Sample Preparation:**

**Preparation of Standard Stock Solution:**

The standard stock solutions of 1000 µg/ml of Dexibuprofen were prepared by weighing 100mg of Dexibuprofen dissolved with methanol in 100 ml volumetric flask and made up to the volume.

**5. Application of Sample:**

Generally 1.0-5.0µg/spot is most satisfactory for HPTLC application of the sample & standard as a band gives better separation, equal $R_f$ values, and less spot broadening .The sample is applied a series of 1µg, 2µg, 3µg, 4µg & 5µg/spot Standard of Dexibuprofen solution were loaded as 4mm band length in the 6 x 10 Silica gel $60F_{254}$ TLC plate using 100µl Hamilton syringe and CAMAG-LINOMAT-5 instrument.

**6. Spot Development :**

The Sample and standards loaded plate was kept in Twin trough chamber 10 x 10cm with respective mobile phase up to 15min for Chamber saturation. After completion of chamber saturation, the plate was kept in mobile phase for development up to 90mm.

**7. Photo-Documentation:**

The developed plate was dried by hot-air to evaporate solvents from the plate and the plate was kept in Photo-documentation chamber. The images of developed plate were captured at white light, UV 254nm and UV 366nm using CAMAG-REPROSTAR-3 instrument.

**8. Scanning:**

The developed plate was scanned in UV 254nm wavelength for Dexibuprofen using CAMAG-TLC SCANNER-3 instrument. The Baseline display, Peak densitogram & Peak table of each track were obtained. The Dexibuprofen content present in the loaded sample was evaluated by Peak assignment with 5 level Dexibuprofen standards.

**9. Spectrum scanning**:

The assigned peaks of standards and sample were scanned in spectrum of UV region (200nm-400nm) and found the λmax value of Dexibuprofen.

**10. Linearity:**

Aliquots of 1-5 µg/spot of standard solution of Dexibuprofen is applied on the plate with the help of micro litre syringe using an automatic sample applicator. The plates were developed, dried and scanned densitometrically at 254 nm. The drug peak-area was calculated for each concentration level and a graph was plotted of drug concentration against the peak area and shown in (Fig: 18.6 – 18.12 Table: 14). Calibration parameters are given in Table: 15.

**HPTLC CHROMATOGRAMS FOR DEXIBUPROFEN:**

**Fig: 18 Daylight before mobile phase run**


**Fig: 18.1 UV 366nm before mobile phase run**

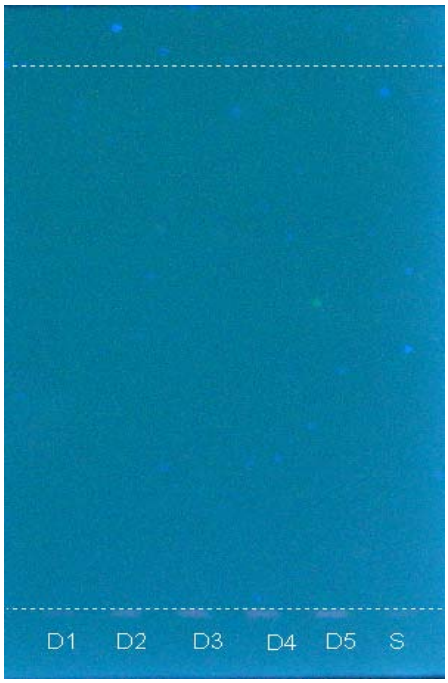



**Fig: 18.2 UV 254nm before mobile phase run**

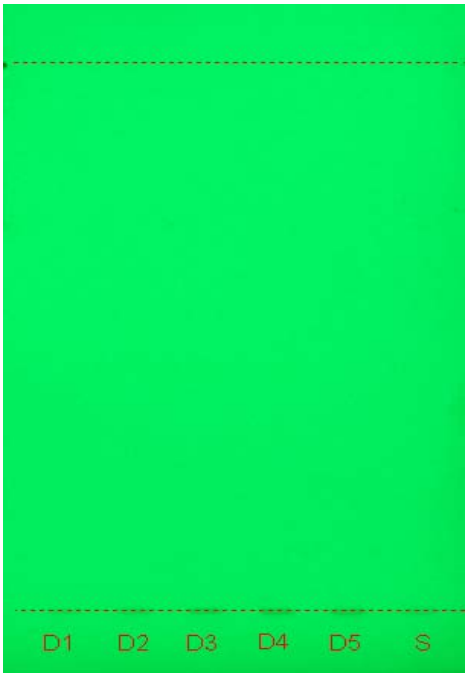


**Fig: 18.3 Daylight after mobile phase run**


**Fig: 18.4 UV 366nm after mobile phase run**

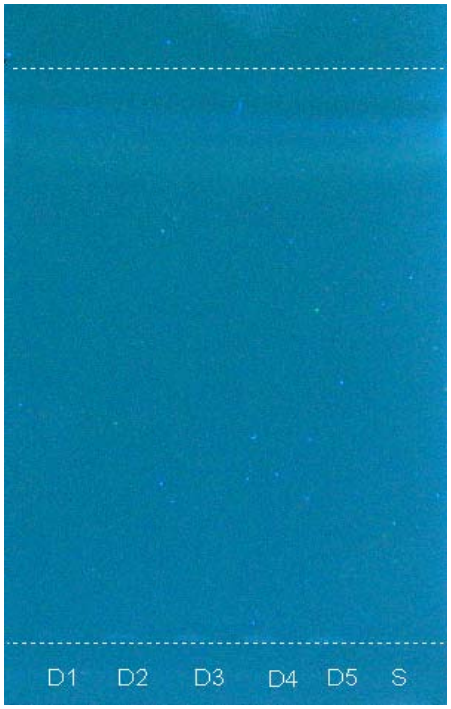



**Fig: 18.5 UV 254nm after mobile phase run**

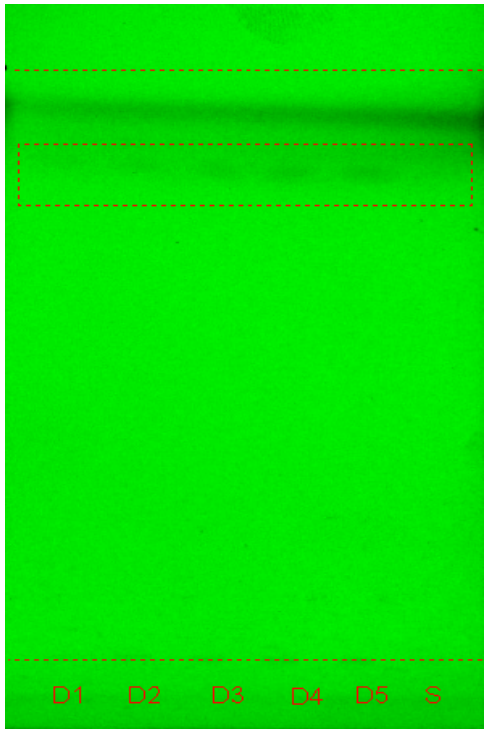


**Fig: 18.6 DEXIBUPROFEN DENSITOGRAM – 1 µg**

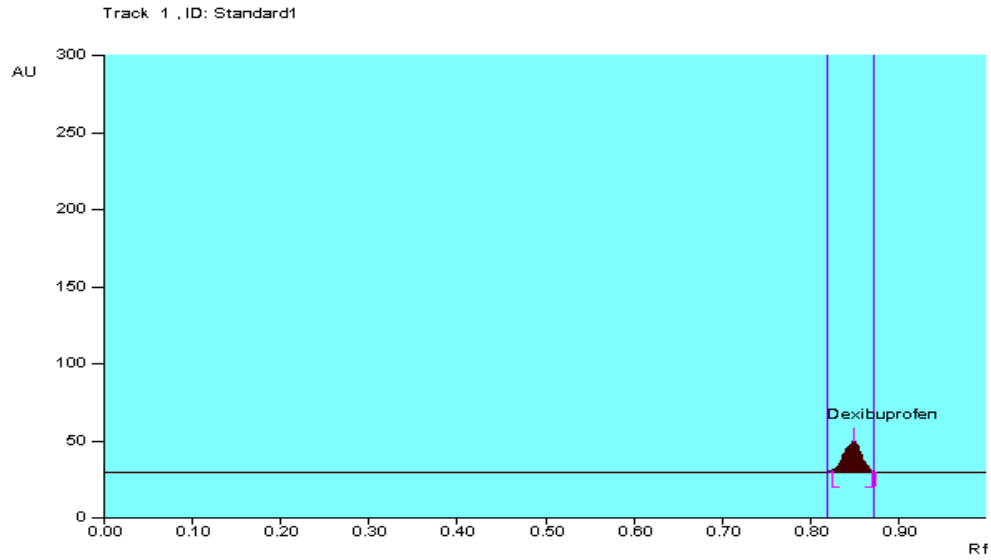



**Fig: 18.7 DEXIBUPROFEN DENSITOGRAM – 2 µg**

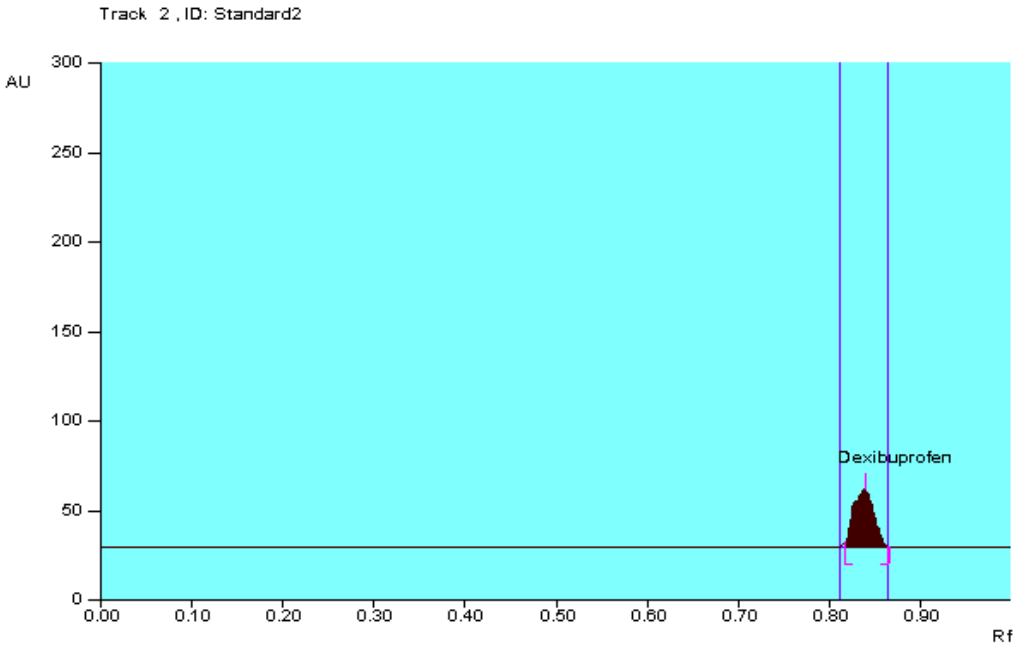


**Fig: 18.8 DEXIBUPROFEN DENSITOGRAM – 3 µg**

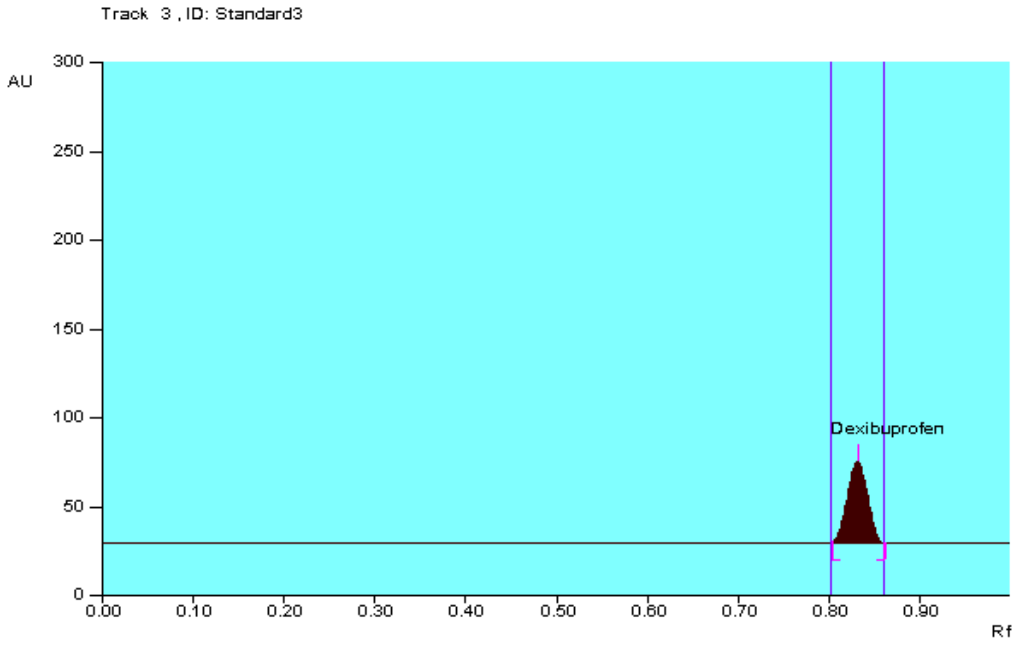



**Fig: 18.9 DEXIBUPROFEN DENSITOGRAM – 4 µg**

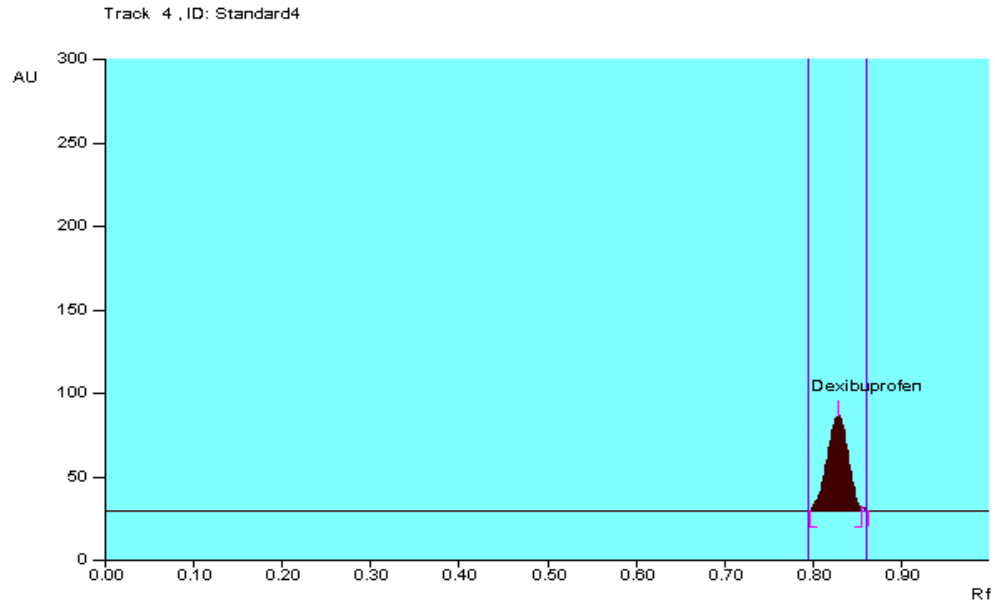


**Fig: 18.10 DEXIBUPROFEN DENSITOGRAM – 5 µg**

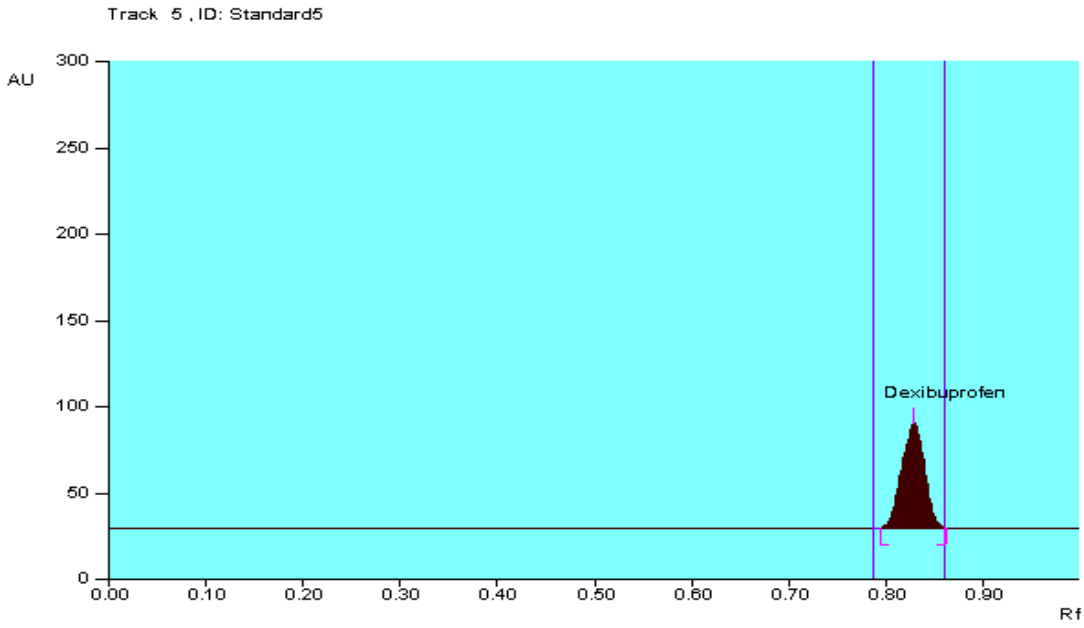
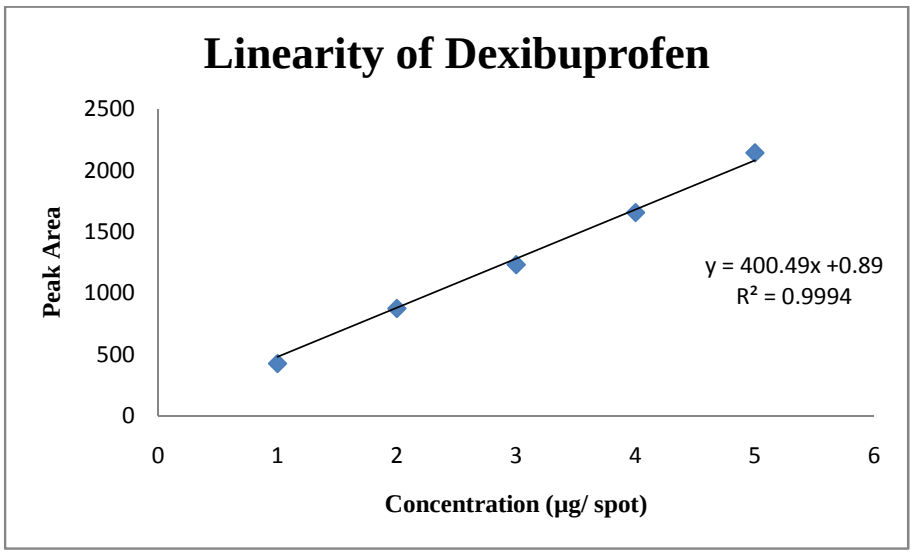



**Fig: 18.12 Calibration Curve of Dexibuprofen at 254 nm**



**ANALYSIS OF FORMULATION:**

**Preparation of Sample Solution:**

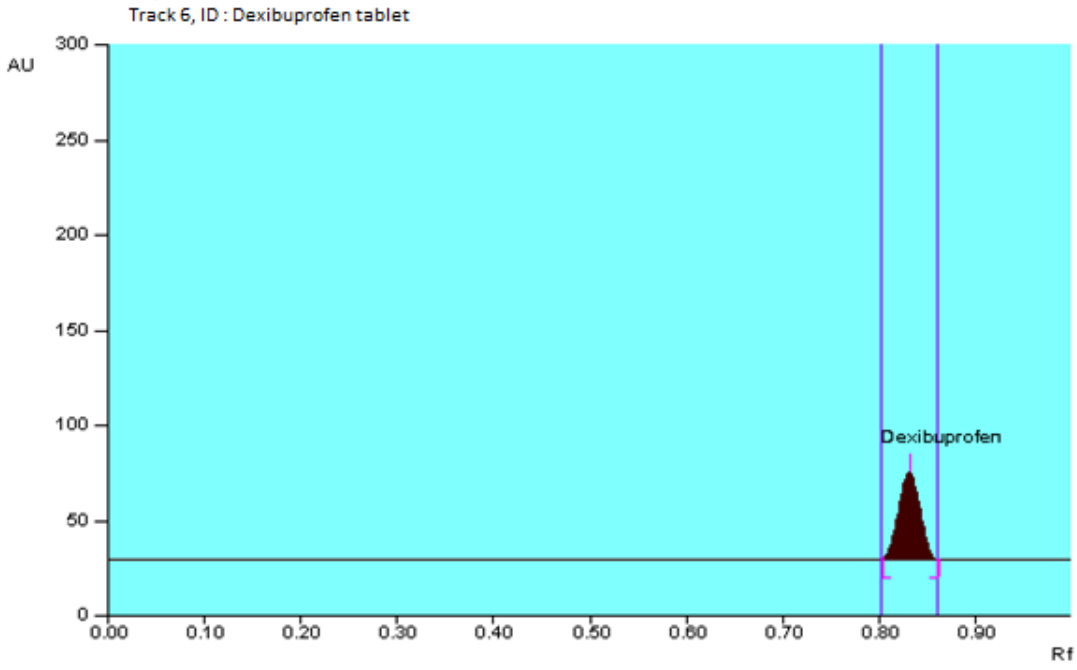
Twenty tablets were powdered and weighed equivalent to 100 mg of Dexibuprofen which is transferred in to a100ml volumetric flask and extracted with methanol the extract was filtered through Whatman filter paper No.41 and residue washed with methanol and made up to 100ml with methanol. Aliquot of 3µg solution of tablet formulation were applied and plate was developed with mobile phase.

**Assay:**

The sample solutions were spotted along with the standard to check the specificity. Spotted 3µg of sample solution allowed to develop in appropriate mobile phase and detect the spots as described earlier. From the peak area recorded the amount of the drug in the formulation was determined (Table: 13).

**ANALYSIS OF FORMULATION:**

**Fig: 18.11 Scanning Peak densitogram of DEXIBUPROFEN sample**

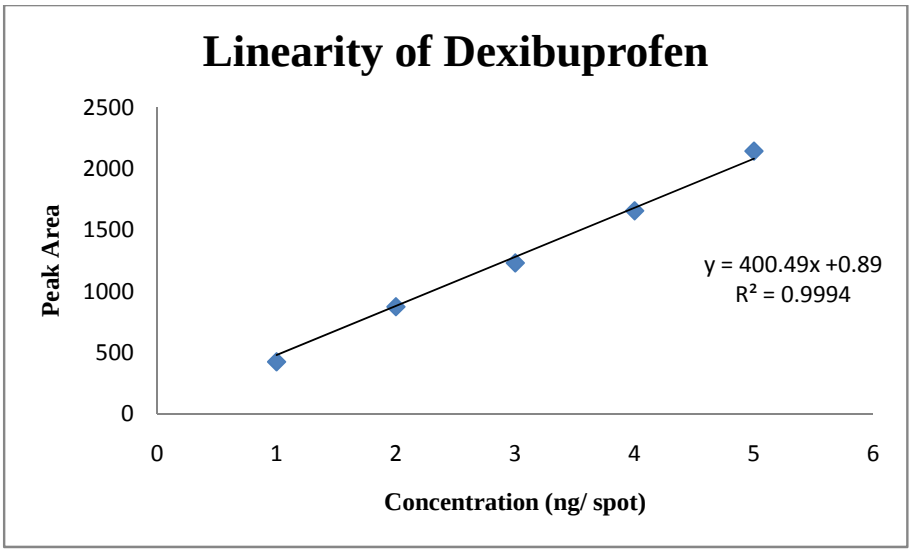


**METHOD VALIDATION:**

**1. Linearity:**

Aliquots of 1-5 µg/spot of standard solution of Dexibuprofen is applied on the plate with the help of micro liter syringe using an automatic sample applicator. The plates were developed, dried and scanned densitometrically at 254 nm. The drug peak-area was calculated for each concentration level and a graph was plotted of drug concentration against the peak area and shown in Table: 14, Fig.18.12. Calibration parameters are given in Table: 15.

**Fig: 18.12 Calibration Curve of Dexibuprofen at 254 nm**




**2. Accuracy:**

Accuracy of the developed method was confirmed by doing a recovery study as per ICH guidelines at three different concentration levels (80%, 100% and 120%) by replicate analysis (n=3). Standard drug solutions were added to a preanalyzed sample solution, and then percentage of drug content was calculated. The results of the accuracy study are reported in Table 16. From the recovery study, it was clear that the method is very accurate for quantitative estimation of Dexibuprofen in tablet dosage form because all the statistical results were within the acceptance range (i.e., % RSD <2.0).

**3. Precision:**

The precision of the method (system reproducibility) was assessed by spotting 3µl of drug solution six times on a TLC plate, followed by development of plate and recording the peak area for 6 spots. The % RSD for peak area values of Dexibuprofen was found to be 0.86%. The results were shown in Table-17.

The method reproducibility (intra-day precision) was determined by analyzing standard solution in the concentration range of 1 µg/spot to 5 µg/spot of drug for 3 times on the same day and inter-day precision was determined by analysing corresponding standards daily for 3 day over a period of one week. The intra-day and inter-day coefficients of variation (%RSD) are in range of 0.13 to 1.6 and 0.14 to 0.40, respectively. The results were shown in Table- 17a, 17b.

## 8. RESULT AND DISCUSSION

In the present work a new method development and validation was carried out for the estimation of dexibuprofen tablets in pharmaceutical dosage form by RP-HPLC and HPTLC method.

**METHOD DEVELOPMENT AND VALIDATION OF DEXIBUPROFEN TABLETS ASSAY BY RP-HPLC:**

**METHOD DEVELOPMENT:**

- To develop an effective method for the analysis of the dexibuprofen in pharmaceutical formulations. Preliminary tests were performed in order to select adequate and optimum conditions.
- Parameters such as detection of wavelength, ideal mobile phase and their proportions, optimum pH and concentration of standard solution were studied.
- Preliminary studies involved experiments with different $C_{18}$ columns were conducted to achieve best separation. $C_{18}$ columns such as Hypersil BDS $C_{18}$ was tried.
- After reviewing the results it was found that the peak shape, retention time, resolution, tailing factor, and column efficiency are good with Hypersil BDS $C_{18}$ (150 x4.6mm,5.0µm) column was selected.
- During the stage of method development mixture of mobile phases was tried and the mobile phase comprising of acetonitrile: water (60: 40 % v/v) with 1 ml of glacial acetic acid and 0.2 ml of triethylamine. Adjust the pH to 5.0 ± 0.05 using triethylamine or glacial acetic acid by isocratic elution, Dexibuprofen showed a retention time of 4.5 ± 0.3 minutes respectively, with the flow rate of 1.0ml/ min and a detection wave length of 264nm with injection volume of 10µl at ambient temperature. Therefore, the tailing factor, resolution and peak shape was found to be good in the finally reported conditions for the dexibuprofen. The chromatograms of trial methods were as shown in Fig.No:2-4 and optimized method were as shown in Fig. No: 5-7. The assay results were as shown in Table No: 1.

**RESULTS OF TRIAL- 1:**

In this trial-1 Dexibuprofen shows good absorbance at 264 nm, so it was selected as wavelength of detection. The mobile phase consisting of Acetonitrile: water (50: 50%v/v), Column used as Hypersil BDS column (150 × 4.6mm, 5µ), Flow rate 1.0 ml were tried to achieve the separation. But it was found that multiple peaks are present in the chromatogram.

**RESULTS OF TRIAL – 2:**

Next trial was made with changing the mobile phase ratio consisting Acetonitrile: water (55: 45% v/v), column used as Hypersil BDS column (150 × 4.6mm, 5µ), Flow rate 1.0 ml in this trial chromatogram shows peak tailing was observed and the peak shape and height was not found to be good.

**RESULTS OF TRIAL – 3:**

The mobile phase of Acetonitrile: water was tried and the chromatogram was recorded at 264 nm at the ratio of (60:40% v/v) was selected as the ideal ratio for the estimation of dexibuprofen and add 0.1 ml of triethylamine. But the chromatogram shows the retention time of the peak was observed at 4.8 min and a small humb was present.

**RESULTS OF FINAL OPTIMIZED METHOD:**

Keeping the mobile phase ratio of Acetonitrile: water (60:40%v/v) and add the peak modifiers such as 1 ml of glacial acetic acid and 0.2 ml of triethylamine and adjust the pH to 5.0± 0.05. The chromatogram was recorded to get a sharp symmetrical peak with good retention time.

**Table No: 1 Table showing Assay Results of Dexibuprofen tablets**

| Name | Retention time (min) | Area | USP Tailing | USP Plate Count | Amount of drug (mg) | Amount of drug (%) |
|---|---|---|---|---|---|---|
| Dexibuprofen | 4.456 | 2213622 | 1.2 | 485 | 298.3 | 99.4 |

**METHOD VALIDATION:**

After method development, the validation of the current method has been performed. The objective of method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines.

The method was validated for linearity, accuracy, precision, range, specificity, solution stability, filter interference study, and force degradation.

- System suitability tests were carried out by five replicate injections, the peak area and retention time was calculated. The %RSD of peak area and the retention time was within the limit of ±2%. This indicates that the method was system suitable.
- The linearity of Dexibuprofen were determined and the linearity based on the area observed in the range of 20-100 µg /ml. The regression co-efficient value was found to be 0.9999.
- The accuracy was studied by the recovery studies. Known amount of dexibuprofen spiked with placebo at about 80%, 100% and 120% of working concentration in triplicate and analysed as per testing procedure. The percentage recovery was Dexibuprofen was found to be in the range of 99.80 – 100.27 %.

## 4. RESULTS

### 4.1 PRECISION:

#### 4.1.1 SYSTEM PRECISION:

Five replicate injections of standard solution were injected. The mean and percentage relative standard deviation (% RSD) for peak areas of dexibuprofen were calculated. The results are tabulated in table-2.

**Percentage relative standard deviation (%RSD) for peak areas of dexibuprofen is not more than 2.0.**

**SYSTEM PRECISION**

**TABLE -2**

| **INJECTION NO (std conc: 100µg/ml)** | **PEAK AREA** |
|---|---|
| 1. | 2264976 |
| 2. | 2271195 |
| 3. | 2273532 |
| 4. | 2283832 |
| 5. | 2267417 |
| Mean | 2272190 |
| Percentage relative standard deviation (%RSD) | 0.3 |

**CONCLUSION:**

Percentage relative standard deviation (%RSD) value indicates an acceptable level of precision of the analytical system for the determination of assay of dexibuprofen tablets.

**4.1.2 METHOD PRECISION**

Six samples of 300 mg tablets prepared and analysed as per testing procedure. The percentage of assay in six samples and percentage relative standard deviation (%RSD) were calculated. The results are tabulated in table - 3.

**Percentage relative standard deviation (%RSD) for percentage of assay is not more than 2.0.**

**METHOD PRECISION**

**TABLE – 3**

| **SAMPLE NO ( 96µg/ml)** | **PERCENTAGE ASSAY** |
|---|---|
| Sample 1 | 101.8 |
| Sample 2 | 120.1 |
| Sample 3 | 99.6 |
| Sample 4 | 98.1 |
| Sample 5 | 101.6 |
| Sample 6 | 103.2 |
| Average | 101.1 |
| Percentage relative standard deviation (%RSD) | 1.8 |

**CONCLUSION:**

Percentage relative standard deviation (%RSD) value indicates an acceptable level of precision of the analytical method for the determination of assay of dexibuprofen tablets.

**4.1.3 INTERMEDIATE PRECISION (RUGGEDNESS):**

Ruggedness of the method was verified by analysing the six samples of 300 mg tablets of same batch which was used for method precision as per testing procedure. This study was performed by different analyst using different instrument and different column on different day. Calculated the Percentage assay and percentage relative standard deviation (%RSD) for six assay results. The results are tabulated in table – 4.

**Percentage relative standard deviation (%RSD) for assay is not more than 2.0**

**INTERMEDIATE PRECISION**

**TABLE - 4**

| SAMPLE NO (96µg/ml) | PERCENTAGE OF ASSAY | |
|---|---|---|
| | ANALYST – 1 | ANALYST – 2 |
| Sample 1 | 101.8 | 101.3 |
| Sample 2 | 120.1 | 100.6 |
| Sample 3 | 99.6 | 100.1 |
| Sample 4 | 98.1 | 101.1 |
| Sample 5 | 101.6 | 101.1 |
| Sample 6 | 103.2 | 102.2 |
| Average | 101.1 | 101.1 |
| Percentage relative standard deviation (%RSD) | 1.8 | 0.7 |

**CONCLUSION:**

Percentage relative standard deviation (%RSD) value indicates an acceptable level of ruggedness of the analytical method for the determination of assay of dexibuprofen tablets.

**3.2 SPECIFICITY:**

Blank, placebo, standard , sample solution (unspiked) and sample solution spiked with known impurities at 1% level were injected into the HPLC system. There was no interference from the blank and placebo at the retention time of dexibuprofen peak. Peak purity data reveals that dexibuprofen peak was homogenous and there were no co-eluting peaks at the retention time of dexibuprofen peak. Calculated the percentage difference between the mean assay of unspiked and spiked sample with respect to unspiked sample. The results are tabulated in table – 5a. The peak purity data of dexibuprofen peak from standard , sample (unspiked) and spiked sample are summarized in table – 5b.

**ACCEPTANCE CRITERIA:**

i. No peaks elutes at the retention time of dexibuprofen in blank and placebo.
ii. The percentage difference between the assay values of spiked and unspiked sample is not more than ± 2.0 with respect to unspiked sample.
iii. Peak purity passes.

**SPECIFICITY OF DEXIBUPROFEN**

**TABLE – 5 A**

| Mean % Assay Of Sample (Unspiked) | Mean % Assay Of Spiked Sample | % Difference |
|---|---|---|
| 98.5 | 100.4 | 1.5 |

**TABLE – 5 B: PEAK PURITY OF DEXIBUPROFEN**

| Peak Name | Sample Type | Purity Angle | Purity Threshold | Purity Flag |
|---|---|---|---|---|
| Dexibuprofen | Standard | 0.289 | 0.516 | No |
| | Sample (unspiked) | 0.286 | 0.524 | No |
| | Spiked sample | 0.425 | 0.495 | No |

**CONCLUSION:**

The method is specific for the determination of assay of dexibuprofen tablets.

**3.3 FORCED DEGRADATION:**

Forced degradation study was carried out by treating the sample under the following conditions.

**a) Degradation By Hydrochloric Acid (Acid Treated Sample):**

Sample was treated with 10 ml of 5N Hydrochloric acid and kept on bench top for 12 hours. Treated sample solution was analysed as per the testing procedure.

**b) Degradation By Sodium Hydroxide (Base Treated Sample):**

Sample was treated with 10 ml of 5N sodium hydroxide and kept on bench top for 12 hours. Treated sample solution was analysed as per the testing procedure.

**c) Degradation By Hydrogen Peroxide (Peroxide Treated Sample):**

Sample was treated with 10 ml of 30% solution of hydrogen peroxide and kept on bench top for 12 hours. Treated sample solution was analysed as per the testing procedure.

**d) Degradation By Thermal (Heat Treated Sample):**

Sample was kept in an oven at 105ºc for about 24 hours. Treated sample was analysed as per the testing procedure.

**e) Degradation By Photo Light [Controlled Condition (Wrapped In Aluminium Foil)]:**

Sample was exposed to light of 1.2 million lux hours in protected condition. Treated sample was analysed as per the testing procedure.

**F) Degradation By Photo Light [Uncontrolled Condition]:**

Sample was exposed to light of 1.2 million lux hours. Treated sample was analysed as per the testing procedure. The results of forced degradation studies are summarized in table – 6.

**Acceptance criteria**

i. Peak purity for main peak passes.
ii. Degradation is not more than 30% in each condition.

**Forced Degradation**

**Table – 6**

| SL. No | Condition | % Assay | % Degradation | Peak Purity Of Dexibuprofen | | |
|---|---|---|---|---|---|---|
| | | | | Purity Angle | Purity Threshold | Purity Flag |
| 1. | Untreated Sample | 100.8 | - | 0.293 | 0.519 | No |
| 2. | Acid Stressed Sample | 89.9 | 10.9 | 0.314 | 0.539 | No |
| 3. | Alkali Stressed Sample | 98.7 | 2.1 | 0.333 | 0.561 | No |
| 4. | Peroxide Stressed Sample | 99.0 | 1.8 | 0.319 | 0.536 | No |
| 5. | Thermal Stressed Sample | 79.8 | 21.0 | 0.380 | 0.610 | No |
| 6. | Photo Light Stressed Sample Controlled Condition | 99.8 | 1.0 | 0.331 | 0.567 | No |
| 7. | Photo Light Stressed Sample Uncontrolled Condition | 98.4 | 2.4 | 0.286 | 0.516 | No |

**Conclusion:**

The method is stability indicating for determination of assay of dexibuprofen tablets.

**3.4 Linearity**

The linearity of dexibuprofen was performed in the range of 20 µg/ml to 100 µg/ml (20% to 100% of working concentration). A graph was plotted with concentration ( in µg/ml) on x-axis and peak area on y- axis. Slope, y- intercept, correlation coefficient (r- value) were determined.

The results are tabulated in table – 7.

**Acceptance criteria:**

The correlation coefficient (r) value is not less than 0.99.

**LINEARITY**

**TABLE – 7**

| Level | Concentration In µg/ml | Peak Area |
|---|---|---|
| 20% | 20 | 477056 |
| 40% | 40 | 922708 |
| 60% | 60 | 1416756 |
| 80% | 80 | 1903940 |
| 100% | 100 | 2339886 |
| Slope | 23534 | |
| y – intercept | 0.7619 | |
| Correlation coefficient | 0.9999 | |

**Conclusion:**

The detector response is directly proportional to concentration ranging 20µg/ml to 100µg/ml (20% to 100% of working concentration).

**3.5 Accuracy (Recovery):**

The accuracy of an analytical method may be defined as the closeness of the test results obtained by the method to the true value. It is the measure of the exactness of the analytical method developed. Accuracy may often express as present recovery by the assay of a known amount of analyte added.

The accuracy was demonstrated by preparing recovery sample. Known amount of dexibuprofen spiked with placebo at about 80%, 100% and 120% of working concentration in triplicate and analysed. Prepared each level in the triplicates and the average value taken to calculate the recovery. The percentage recovery was calculated from the amount found and actual amount added.

The results are tabulated in table – 8.

**Acceptance criteria:**

Percentage recovery at each level is in between 98.0 and 102.0.

Percentage relative standard deviation (%RSD) is not more than 2.0 at each level.

**Accuracy (Recovery)**

**Table – 8**

| Level | Actual Amount Added (in mg) | Amount Found (in mg) | % Recovery | Mean (n =3) | % RSD |
|---|---|---|---|---|---|
| Level – 1 (80%) | 240.43 | 240.58 | 100.06 | 100.27 | 0.4 |
| | 238.72 | 240.92 | 100.09 | | |
| | 239.56 | 241.15 | 100.66 | | |
| Level – 2 (100%) | 298.84 | 300.15 | 100.43 | 99.99 | 0.3 |
| | 300.03 | 299.87 | 99.94 | | |
| | 301.25 | 300.12 | 99.62 | | |
| Level – 3 (120%) | 361.36 | 360.22 | 99.68 | 99.80 | 0.1 |
| | 360.05 | 359.13 | 99.74 | | |
| | 360.98 | 361.01 | 100.00 | | |

**CONCLUSION:**

The analytical method meets the pre-established acceptance criteria for accuracy study as per protocol. Hence the method is accurate for the determination of assay of Dexibuprofen tablets.

**3.6 RANGE:**

Range inferred from the data of linearity, accuracy and precision experiments.

**3.7 SOLUTION STABILITY:**

Stability of analytical solution was verified by analysing the standard solution and sample solution of 300 mg tablets initially and also at different time intervals as mentioned below by storing in sample compartment of HPLC instrument at25ºC (ambient). Calculated the percentage relative standard deviation (%RSD) for peak areas of dexibuprofen in standard and sample. The results are tabulated in table – 9.

**ACCEPTANCE CRITERIA:**

Percentage relative standard deviation (% RSD) is not more than 2.0 for peaks areas of standard and sample.

**SOLUTION STABILITY**

**Table – 9**

| Time In hrs. | Standard Solution | | Sample Solution | |
|---|---|---|---|---|
| | Peak Area | % RSD | Peak Area | % RSD |
| Initial | 2272190 | - | 2223022 | - |
| 4 | 2271777 | 0.01 | 2217590 | 0.2 |
| 8 | 2282898 | 0.3 | 2218966 | 0.1 |
| 12 | 2248931 | 0.6 | 2195335 | 0.6 |
| 16 | 2285267 | 0.6 | 2183930 | 0.8 |
| 20 | 2279895 | 0.6 | 2222586 | 0.7 |
| 24 | 2278672 | 0.5 | 2232002 | 0.8 |

**CONCLUSION:**

As the percentage relative standard deviation (% RSD) for standard and sample solution were within the acceptance criteria, hence it was concluded that standard solution and filtered sample solutions are stable up to 24 hours at 25ºC(ambient).

**3.8 FILTER INTERFERENCE STUDY:**

Filter interference study was performed on sample solution of 300 mg tablets. Prepared sample as per testing procedure filtered the solution through 0.45µ nylon filter, analysed the sample and the result compared with centrifuged sample result.

The results are tabulated in table – 10.

**Acceptance criteria:**

The percentage difference between centrifuged and filtered sample results is not more than ± 2.0 with respect to centrifuged sample results.

**FILTER INTERFERENCE STUDY**

**Table – 10**

| Sample Name | Percentage Assay | Percentage Difference |
|---|---|---|
| Centrifuged sample | 101.9 | - |
| Filtered sample | 101.8 | 0.1 |

**CONCLUSION:**

The percentage difference value within the acceptance limit for filtered sample. Hence 0.45µm nylon filters suitable for filtering the sample solution of dexibuprofen tablets.

**3.9 ROBUSTNESS:**

Robustness of the method verified by deliberately varying the following instrumental conditions.

a) By changing the flow rate by ± 10%.
b) By changing the wavelength by ± 2 nm.
c) By changing the organic content in mobile phase by ± 2% absolute.
d) By changing the mobile phase pH by ± 0.2 units.

System suitability was evaluated in each condition and 300 mg tablet sample was analysed in triplicate. The results were compared with the method precision data and tabulated in table – 11.

**ACCEPTANCE CRITERIA:**

Overall percentage relative standard deviation (%RSD) for assay of each change condition and method precision results is not more than ± 2.0.

**ROBUSTNESS**

**TABLE - 11**

| SL. No | Minus wavelength (218 nm) | Plus wavelength (222 nm) | Minus flow rate (0.9 ml/min) | Plus flow rate (1.1 ml/ min) | Minus organic (58%) | Plus organic (62% ) | Minus pH (4.8) | Plus pH(5.2) |
|---|---|---|---|---|---|---|---|---|
| 1 | 98.6 | 98.6 | 100.9 | 99.2 | 98.4 | 98.3 | 98.2 | 98.1 |
| 2 | 98.5 | 98.5 | 100.2 | 99.3 | 101.3 | 100.7 | 100.8 | 100.2 |
| 3 | 100.3 | 100.1 | 101.2 | 98.8 | 101.2 | 101.3 | 100.7 | 99.9 |
| Overall Mean | 100.4 | 100.4 | 101.0 | 100.4 | 100.8 | 100.7 | 100.7 | 100.5 |
| Overall % RSD | 1.8 | 1.8 | 1.5 | 1.8 | 1.7 | 1.7 | 1.7 | 1.8 |

**CONCLUSION:**

The method is robust for change in flow rate, change in organic content in mobile phase, change in buffer pH and change in wave length.

**Table: 12 Results of Assay Method Validation of Dexibuprofen Tablets**

| S.N0 | Parameters | Observation | Acceptance Criteria | Passes/ Fail |
|---|---|---|---|---|
| 1.<br><br>1.1<br>1.2<br>1.3 | Precision<br><br>System Precision<br>Method Precision<br>Intermediate Precision | <br><br>0.3<br>1.8<br>1.3 | Percentage relative standard deviation (%RSD) is not more than 2.0 | Passes |
| 2. | Specificity | No peak elute | No peak elutes at the retention time of main peak in the blank and placebo | Passes |
| 3. | Forced Degradation<br>a. Degradation By Hydrochloric Acid<br>b. Degradation By Sodium Hydroxide<br>c. Degradation By Hydrogen Per- Oxide<br>d. Degradation by Thermal Heat.<br>e. Degradation By Exposing Light | <br>10.9<br>2.1<br>1.8<br>21.0<br>1.0 | Degradation is not more than 30 % in each condition | Passes |
| 4. | Linearity | 0.9999 | Correlation coefficient is not less than 0.99 | Passes |
| 5. | Accuracy | The percentage recovery was found between 99.80 – 100.27% | The percentage Recovery at each level is between 98.0-102.0% | Passes |
| 6. | Solution Stability | Well within the limit | %RSD is not more than 2.0 | Passes |
| 7. | Filter Interference | 0.1 | The percentage difference is not more than 2.0 | Passes |
| 8. | Robustness | Well within the limit | Overall percentage Relative standard deviation (%RSD) is not more than 20 | Passes |

## METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF DEXIBUPROFEN IN TABLET DOSAGE FORM BY HPTLC

### HPTLC METHOD DEVELOPMENT:

- For HPTLC analysis, initially various mobile phase and stationary phases were tried in attempts to obtain the best separation and resolution of Dexibuprofen.
- The mobile phase consisting Toluene: Ethyl acetate: Glacial acetic acid in the proportion of (6: 3: 1% v/v/v) selected that gave satisfactory separation and gave well resolved peaks for Dexibuprofen.
- Dexibuprofen exhibit significant absorbance at a wavelength 254nm was selected as a detection wavelength for the determination of dexibuprofen.
- Well defined spots were obtained when the chamber was saturated with mobile phase for 15 min ensures good reproducibility and peak shape. The $R_f$ value for Dexibuprofen was 0.83±0.02.
- The Dexibuprofen showed good linearity in the range of 1-5 µg per spot with coefficient of correlation value 0.9994 for peak area. (chromatogram for Dexibuprofen and linearity densitogram was shown in fig: 18 – 18.11.)
- Spotted 3µg of Dexibuprofen sample solution allowed to develop in appropriate mobile phase and detect the spots as described. From the peak area recorded the amount of the drug in the formulation was determined (Table: 13).

**Table -13 Analysis of Formulations**

| S.No. | Drug | Label Claim(mg) | Amount found (mg) | Assay %RSD |
|---|---|---|---|---|
| 1. | Dexibupropfen | 300 | 296.85 | 98.95±0.69 |

**METHOD VALIDATION:**

After method development, the validation of the current method has been performed. The objective of method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, accuracy, precision.

**1. Linearity:**

For linearity study, aliquots of 1.0, 2.0, 3.0, 4.0, and 0.5 µg from Dexibuprofen standard stock solution was applied on TLC. The plates were developed, dried and scanned densitometrically at 254 nm. The drug peak-area was calculated for each concentration level and a graph was plotted of drug concentration against the peak area and shown in (Table: 14, Fig.18.12). Calibration parameters are given in Table: 15.

**Table - 14: Linearity of Dexibuprofen**

| SL. No | Standard concentration µg/ spot | Peak Area |
|---|---|---|
| 1 | 1.0 | 425.3 |
| 2 | 2.0 | 874.3 |
| 3 | 3.0 | 1231.6 |
| 4 | 4.0 | 1656.2 |
| 5 | 5.0 | 2143.0 |

**Table -15 Calibration Parameters for DEXIBUPROFEN**

| Parameters | Dexibuprofen |
|---|---|
| Linearity Range(µg/Spot) | 1-5 |
| Slope | 400.49 |
| Intercept | 0.89 |
| Regression Co-Efficient | 0.9994 |

**2. Accuracy**:

The proposed method when used for extraction and subsequent estimation of drug from tablet dosage form after over spotting with 80, 100 and 120 % of additional drug; mean recovery is within acceptable limits, indicating the method is accurate and afforded recovery of 98.82 – 99.69 %. It showed very low % RSD of peak area of Dexibuprofen (Table: 16).

**Table -16: Recovery studies for Dexibuprofen (n=3)**

| Label claim (mg / tablet) | Recovery level | Amount added (mg) | Amount recovered (mg) ± % RSD | % Recovery |
|---|---|---|---|---|
| 300 | 80 | 240 | 238.16±0.42 | 98.82 |
| 300 | 100 | 300 | 298.68±0.85 | 98.67 |
| 300 | 120 | 360 | 358.90±0.59 | 99.69 |

**3. Precision:**

The repeatability and intermediate precision were studied (Table-3 and -4 respectively). Repeatability It showed very low % RSD values of intra-day and inter-day are in range of 0.13 to 1.6 and 0.24 to 1.6, Dexibuprofen respectively. The results were shown in Table-17, 17a, and 17b.

**Table – 17: Precision of Dexibuprofen**

| S. No | Concentration (µg/ spot) | Peak Area |
|---|---|---|
| 1. | 3 | 1212.1 |
| 2. | 3 | 1218.4 |
| 3. | 3 | 1209.6 |
| 4. | 3 | 1188.4 |
| 5. | 3 | 1211.8 |
| 6. | 3 | 1203.5 |
| Mean | - | 1207.3 |
| **Percentage Relative Standard Deviation** | - | 0.86 |

**Table – 17a: Intra-day Precision of Dexibuprofen**

| S. No | Concentration (µg / spot) | Area | Mean | Standard Deviation | % RSD |
|---|---|---|---|---|---|
| 1. | 1 | 411.3 | | | |
| 2. | 1 | 400.9 | 403.7 | 6.65 | 1.6 |
| 3. | 1 | 398.9 | | | |
| 1. | 3 | 1213.9 | | | |
| 2. | 3 | 1210.3 | 1211.9 | 1.8 | 0.15 |
| 3. | 3 | 1211.6 | | | |
| 1. | 5 | 2146.2 | | | |
| 2. | 5 | 2143.9 | 2146.6 | 2.9 | 0.13 |
| 3. | 5 | 2149.8 | | | |

**Table – 17b: Inter-day Precision of Dexibuprofen**

| S. No | Concentration (µg / spot) | Area | Mean | Standard Deviation | % RSD |
|---|---|---|---|---|---|
| 1. | 1 | 400.4 | | | |
| 2. | 1 | 399.6 | 399.1 | 1.6 | 0.40 |
| 3. | 1 | 397.3 | | | |
| 1. | 3 | 1205.1 | | | |
| 2. | 3 | 1201.3 | 1201.5 | 3.40 | 0.28 |
| 3. | 3 | 1198.3 | | | |
| 1. | 5 | 2140.7 | | | |
| 2. | 5 | 2141.5 | 2142.9 | 3.2 | 0.14 |
| 3. | 5 | 2146.6 | | | |

**Table – 18 DEXIBUPROFEN HPTLC VALIDATION PARAMETERS**

| S.No | Parameters | Dexibuprofen |
|---|---|---|
| 1. | $R_f$ | 0.83±0.008 |
| 2. | Linearity (µg/ spot) | 1 – 5 |
| 3. | Correlation coefficient ($r^2$) | 0.9994 |
| 4. | Accuracy (% Recovery)<br>(n = 3) %RSD<br>1. 80 %<br>2. 100%<br>3. 120% | <br><br>98.82±0.42<br>98.67±0.85<br>99.69±0.59 |
| 5. | Precision (%RSD, n = 6)<br>Intra-day( n = 3) (%RSD)<br>Inter-day ( n = 3) (%RSD) | 0.86<br>0.17 – 1.5<br>0.13 – 1.6 |

## 9. CONCLUSION

In the present study a validated isocratic HPLC method has been developed for the determination of Dexibuprofen in tablet dosage forms. The analytical procedure is validated as per ICH guidelines and shown to be accurate, precise and specific. The mobile phase and the sample preparation are simple and the analysis time is short to prepare. The recoveries in formulations were in good agreement with their respective label claims. Its chromatographic run time of 10 min allows the analysis of a large number of samples in a short period of time. Therefore, it is suitable for the routine analysis of Dexibuprofen in tablet dosage forms. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as LC-MS/MS or GC-MS/MS that are complicated, costly, and time consuming rather than a simple HPLC method.

The result of analysis of Dexibuprofen tablet dosage forms by the proposed HPTLC method was developed and validated as per ICH guidelines and the method was found to be simple, precise, accurate and highly reproducible, reliable thus can be used for determination of Dexibuprofen. The present recovery obtained was 98.82 – 99.69 % indicates no interference from the common excipients in the tablet formulations. This method can be used for the routine analysis of the Dexibuprofen in tablet dosage form.

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